Is there utility to genetic information in elite sport?

by

Craig Pickering

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ABSTRACT

Variation between individuals in response to a stimulus is a well-established phenomenon. This thesis discusses the drivers of this inter-individual response, identifying three major determinants; genetic, environmental, and epigenetic variation between individuals. Focusing on genetic variation, the thesis explores how this information may be useful in elite sport, aiming to answer the question "Is there utility to genetic information in elite sport?" The current literature was critically analysed, with a finding that the majority of exercise genomics research explains what has happened previously, as opposed to assisting practitioners in modifying athlete preparation and enhancing performance. An exploration of the potential ways in which genetic information may be useful in elite sport then follows, including that of inter-individual variation in response to caffeine supplementation, the use of genetic information to assist in reducing hamstring injuries, and whether genetic information may help identify future elite athletes. These themes are then explored via empirical work. In the first study, an internet-based questionnaire assessed the frequency of genetic testing in elite athletes, finding that around 10% had undertaken such a test. The second study determined that a panel of five genetic variants could predict the magnitude of improvements in Yo-Yo test improvements following a standardised training programme in youth soccer players. The third study demonstrated the effectiveness of a panel of seven genetic variants in predicting the magnitude of neuromuscular fatigue in youth soccer players. The fourth and final study recruited five current or former elite athletes, including an Olympic Champion, and created the most comprehensive Total Genotype Score in the published literature to date, to determine whether their scores deviated significantly from a control population of over 500 non-athletes. The genetic panels were unable to adequately discriminate the elite performers from non-athletes, suggesting that, at this time, genetic testing holds no utility in the identification of future elite performers. The wider utilisation of genetic information as a public health tool is discussed, and a framework for the implementation of genetic information in sport is also proposed. In summary, this thesis suggests that there is great potential for the use of genetic information to assist practitioners in the athlete management process in elite sport, and demonstrates the efficacy of some commercially available panels, whilst cautioning against the use of such information as a talent identification tool. The major limitation of the current thesis is the low sample sizes of many of the experimental chapters, a common issue in exercise genetics research. Future work should aim to further explore the implementation of genetic information in elite sporting environments.

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CONFLICT OF INTEREST STATEMENT

As will become apparent throughout this thesis, I am a (now former) employee of DNAFit Life Sciences, a direct-to-consumer genetic testing company based in the UK. The research and ideas discussed within this thesis represent my personal thoughts, ideas, and conclusions, and at no point was any pressure, financial or otherwise, exerted on me to direct, influence, or edit the findings or conclusions emanating from this thesis.

CHAPTER 1 – INTRODUCTION

1. Why genetic testing?

Coaches, athletes and support staff have long known and understood that there is considerable variation between individuals across a variety of traits. Nowhere is this clearer than at the Olympic Games, where only one athlete can take home the gold medal. Achieving such a performance is often thought of as the accumulation of years of hard work and dedication. However, inherent within this process is the recognition that there are genetic differences between those competing at the highest level, those that don't qualify for the games, and interested observers at home. Whilst we typically think of these genetic differences as underpinning our notion of "talent"—and it is clear that elite athlete status is at least partly heritable (De Moor et al., 2007)—recent research suggests that a number of genetic variants also affect issues such as the response to training (Delmonico et al., 2007, Aleksandra et al., 2016) and the effectiveness of ergogenic aids (Guest et al., 2018; Heibel et al., 2018), while also influencing the needs of each individual athlete in terms of recovery speed (Yamin et al., 2008; Baumert et al., 2016a), nutrient requirements (Ashfield-Watt et al., 2002; Timpson et al., 2010), and injury risk (Collins et al., 2009; Willard et al., 2018).

That there is individual variation in response to a stimulus—termed inter-individual variation—has become of increasing interest from a research perspective in recent times, particularly as measurement technologies have improved. Whilst the majority of research tends to explore the mean efficacy of a given intervention, the use of gross averages often obscures individual differences in the magnitude of response. Historically, this individual response was perhaps considered a frustrating outcome that lowered the effect size of an intervention and simultaneously increased the required sample size (Hecksteden et al., 2015). However, as interest in personalised medicine has grown over the last 30 years, individual responses are viewed as increasingly important. Accordingly, research aimed at identifying inter-individual variation, as well as its underlying causes, has become increasingly prevalent across many domains, including exercise (Bouchard & Rankinen, 2001; Hubal et al., 2005; Karavirta et al., 2011; Pickering & Kiely, 2017a), diet (Minot et al., 2011; Williamson et al., 2018), and medicine (Yuan et al., 2005; Hamburg & Collins, 2010). Although such findings have been examined critically (Senn 2002; Atkinson & Batterham, 2015; Williamson et al., 2017), there remains a consensus that there are real differences between individuals in terms of response to an intervention (Mann et al., 2014; Bonafiglia et al., 2019), and the potential that both knowledge and understanding of the drivers of this response may be used to enhance a variety of interventions (Pickering & Kiely 2018a).

Two experiences from my past have shaped my interest in inter-individual variation. As a professional athlete, I was always looking for anything that could potentially improve my performance by any appreciable margin, and, as such, I became interested in the use of caffeine as a performance enhancer. My journey started aged 18, when I started using a sports drink with 80 mg of caffeine prior to races. Over time, this became two cans, then three, and then I moved onto caffeine tablets as my self-experimentation continued. This journey was not without error, however; on more than one occasion, including a national championship final, I had to withdraw from a race due to issues caused by caffeine.

This trial and error process, consistently refined over my competitive career, eventually culminated in my adoption of the following caffeine strategy; I could consume up to 250 mg of caffeine, split over four individual doses, at 10-minute intervals between 90- and 60-minutes pre-race. If I had two races in close succession, such as heats and final, I could only consume low doses (less than 80 mg) of caffeine between the two; any more and I started to feel nauseated.

What was puzzling to me, as an athlete, was that my training partners and competitors could consume far higher doses of caffeine than I could tolerate, and could do so closer to competition than I. What was more puzzling to me, as a sports science student, was that the scientific literature generally reported that caffeine was typically ergogenic at doses higher than I was consuming. As an example, in a meta-analysis of 40 studies exploring the efficacy of caffeine as a performance enhancer, the effective dose of caffeine ranged from between 3 and 13 mg/kg, with a median of 6 mg/kg (Doherty & Smith, 2004). In comparison, I was consistently consuming less than this dose, with a tolerable ceiling of around 2.5 mg/kg. Similar contrasting results to my experience were reported by Ganio and colleagues (2009) in a systematic review, with 3-6 mg/kg reported as the optimum range of doses to exert ergogenic effects.

The second experience came at the 2008 Olympic Games in Beijing, where I was representing Great Britain in the 100m. Competing at the quarter-final stage, I was drawn in a tough race, including the World Record holder, the current US champion, the European Record holder, and the European number 1 ranked athlete for 2008, with only three athletes able to qualify. Against such long odds, I produced a seasons best performance, running 10.18 seconds, albeit for 5th place. The winner, Usain Bolt, was able to essentially jog to a winning time of 9.92. Reflecting on my lack of progression to the next round in the bowels of the stadium post-race, I was forced to consider whether those who did progress to the semi-final stage trained harder than me—the common narrative—or whether they were more "talented", whatever such talent might be.

My interest in better understanding these two experiences, and not being able to find a satisfactory answer, remained throughout my career. Upon retiring from professional sport in 2014 due to injury, I began work at DNAFit Life Sciences, a direct-to-consumer (DTC) genetic testing company providing customers with information about how their genes might influence their optimal diet and training programmes. This role further piqued my interest in inter-individual variation, and gave me an opportunity of potentially understanding this variation via genetic testing.

However, the use of information gleaned from genetic tests is currently in its infancy, and remains controversial (Webborn et al., 2015; Vlahovich et al., 2017a). As such, whilst research in this area does show that specific genetic variants can influence the response to training (Delmonico et al., 2007, Aleksandra et al., 2016) or an ergogenic aid (Guest et al., 2018, Rahimi 2018), at present there are very few studies exploring the utility of this information as an intervention; i.e., if you know an individual has a specific genetic variant or variants, can you enhance their response to a certain stimulus? If such an approach is possible, it has the potential to revolutionise practice, ensuring a far more personalised approach. Returning to my specific example of caffeine use, I now know that I have a genetic variant associated with a reduced ergogenic response following caffeine ingestion (Womack et al., 2012; Guest et

al., 2018; Rahimi 2018). Furthermore, I have a second genetic variant, associated with increased anxiety and sleep disturbances following caffeine ingestion (Retey et al., 2007; Childs et al., 2008), both issues that plagued me during my career following ingestion of higher doses of caffeine.

As a result, being able to utilise genetic information to enhance training selection and response, or use of an ergogenic aid, would be advantageous. This is true from a population health perspective, where the increased training adaptations brought about by genetically guided training might help improve overall fitness, which has been linked to reductions in both obesity (Blair & Church, 2004) and all-cause mortality (Blair et al., 1999; Kodama et al., 2009). Genetically-guided insights would also likely be useful in sporting populations, where the margins between success and failure are often miniscule. However, the use of genetic information in this way is currently underexplored, with the vast majority of research exploring associations as opposed to interventions.

2. Is there utility to genetic information in elite sport? The structure of the thesis.

To that end, the goal of this thesis is to explore the effective utilisation of genetic information in sport, with the research question of "Is there utility to genetic testing in elite sport?" This exploration will be formed of different sections, utilising a mixture of desktop and empirical studies. The sections are formulated as so:

Section 2 – A review of the literature. Here, I will explore the research currently surrounding inter-individual variation and exercise response heterogeneity, unravelling some of the drivers of this variation in response (Chapters 2 & 3). I will also explore some contemporary methodological issues regarding genetic testing in sport, the need for further invention studies, and an outline of the methods I will be utilising in the empirical studies in a chapter on Methodology (Chapter 4). This exploration is important as it allows a firm understanding of the research literature, including methodological and statistical issues which affect the field of exercise genetics research at present. Identification of these issues allows for the findings of the subsequent two sections to be better contextually framed and interpreted.

Section 3 – Joining the dots. This section will be comprised of a number of theoretical papers exploring the potential use of genetic information to enhance outcomes within sporting contexts. Chapter 5 explores inter-individual variation in the ergogenic effects seen following caffeine consumption in athletes, the drivers of this variation, and how this information might be utilised to enhance the use of caffeine in athletes. Chapter 6 explores how information on *ACTN3* genotype may be used to provide insights into the individual response to training, both in terms of adaptation and recovery, as well as injury risk. Chapter 7 presents a theoretical method by which genetic information might be useful in the prevention of hamstring injuries, a current hot topic within the sports science and medicine sphere (Bourne et al., 2018). Finally, Chapter 8 explores whether genetic information can be utilised to discover talented individuals, including those genetically primed to respond favourably to training. This section serves to discuss the potential use of genetic information in elite sport, before it is explored experimentally in Section 4.

Section 4 – Practical use of genetic information in sport. This section is comprised of empirical data collected as part of my doctorate. Chapter 9 reports the results of an internet-based questionnaire exploring the prevalence of, and attitudes towards, genetic testing within sports, from the perspectives of athletes, coaches, and support staff. Chapter 10 demonstrates the potential use of a genetic panel in predicting the magnitude of improvements in aerobic fitness following a standardised training programme. Chapter 11 explores the use of a genetic panel in predicting the magnitude and time-course of neuromuscular fatigue following a repeated sprint training session. Chapter 12 reports on the genetic data of a cohort of elite athletes, including an Olympic Champion, the first time such data has been reported, and attempts to determine if this information would be useful from a talent identification perspective. The purpose of this section of the thesis is to experimentally explore whether genetic information may be useful in elite sport, both in terms of how widely it is currently used, and how useful it might be.

Section 5 – Is there utility to genetic testing in sport? Here, I pull together the various strands of my research in order to answer the research question, as well as exploring the real world and practical implications of both my work, and genetic testing as a whole. Chapter 13 explores some of the wider applications of genetic testing in terms of health and disease. Chapter 14 explores the potential future research directions in this field, as well as a discussion of which further questions require answering, and, finally, Chapter 15 offers the main conclusions of my doctoral thesis.

With the use of genetic information a hot topic within sports science and exercise medicine at present (Webborn et al., 2015), my hope is that this present thesis provides a useful step forward, both in our understanding of how genetic information might be used in elite sport, as well as providing practical examples where the use of such information may enhance practice. A consistent theme within the earlier parts of this thesis is how, whilst there is a relative abundance of research demonstrating how and why genetic variation affects the individual response to an exercise stimulus, there is far less research focused on utilising this information as a method of enhancing future performance. In seeking to address this knowledge gap, I have aimed to publish large parts of this thesis as academic papers, focusing primarily on how we might best utilise genetic information in elite sport. The five sections of this thesis (including section 1, comprised of the front matter and introduction), seek to build the narrative in a linear manner. Section 2 details where we are right now; what does the research suggest are the drivers of inter-individual variation, and how might this information be useful? Expanding on the traditional literature review format, I have aimed to critically analyse some contemporary issues within the exercise genomics sphere, before identifying the methodology utilised in the thesis. Section 3 then asks what *could* we use genetic information for in elite sport, and then section 4 explores *whether* this information, in practice, is effective—and how widely used it currently is. The final section, section 5, aims to bring all the previous sections together, giving an overview of how this thesis has added to the field, presenting a conceptual framework for the use of genetic information in sport, and identifying limitations, as well as future areas for research meriting further exploration.

SECTION 2 – LITERATURE REVIEW

The content of this section draws on three previously published peer-reviewed papers, along with additional work. The published papers are:

Pickering C, Kiely J. Understanding Personalized Training Responses: Can Genetic Assessment Help? Open Sports Sci J. 2017;10(1).

Pickering C, Kiely J. Exercise genetics: seeking clarity from noise. BMJ Open Sport Exerc Med. 2017:e000309. doi: 10.1136/bmjsem-2017-000309

Pickering C, Kiely J. Do non-responders to exercise exist – and if so, what should we do about them? Sports Med. 2018; https://doi.org/10.1007/s40279-018-01041-1

Section preface:

Whenever humans are subjected to an intervention, there is a variation between individuals in response to that intervention. This is the case when it comes to the use of drugs within medicine (Wang et al., 2011), exercise and dietary changes for weight loss (King et al., 2008), and response to a food, nutrient, or chemical, such as caffeine (Guest et al., 2018), or exercise (Hubal et al., 2005). This variation between individuals – often termed inter-individual variation – is a combination of "true" and "false" variation. "False" variation refers to issues such as measurement error, random biological variation, and regression to the mean (Atkinson & Batterham, 2015). "True" variation is what is left once the "false" variation is removed; it is the manifestation of differences in genotype, environment, and life history that cause variation in response (Mann et al., 2014). This section, which acts as the literature review portion of this thesis, explores the causes of inter-individual variation in greater detail. Chapter 2 is comprised of an overview of the "true" underpinnings of inter-individual variation, and results in the development of a model which can be utilised to explain and aid our understanding of why such variation occurs. Chapter 3 is a closer look at some of the contemporary issues surrounding such inter-individual variation, including a discussion around "false" variation, and how this may impact whether non-responders to exercise actually exist. Additionally, Chapter 3 contains a commentary on whether the findings from sports and exercise genetics studies are real, or potential false-positives, which has clear and important implications for the use of such information in practice. Finally, Chapter 4 presents a brief summary of the methodological challenges within the field of exercise genomics, and identifies the methods utilised in the present thesis.

CHAPTER 2 - INTER-SUBJECT VARIATION IN EXERCISE ADAPTATION: CONTRIBUTING FACTORS & THE POTENTIAL UTILITY OF GENETIC TESTING

1. Introduction

Exercise prescription is often comprised of blanket advice. For example, the American College of Sports Medicine (ACSM) recommend that adults undertake more than 150 minutes of moderate intensity and more than 75 minutes of vigorous intensity cardiovascular exercise per week, along with resistance training on two to three days per week (Garber et al., 2011). Regarding resistance training, the ACSM recommend repetition ranges of 8-12 for novices, and 1-12 for intermediates (Kraemer et al., 2009). Given these recommendations, one might think that exercise response is standardised across individuals, or at least tightly distributed around the mean. However, a wide range of variation exists in exercise adaptation between subjects (Bouchard & Rankinen, 2001; Hubal et al., 2005; Mann et al., 2014), meaning that there will be a varied response to the typical guidelines, with some individuals seeing larger than average improvements, and some seeing little or no improvements.

Given that this variation occurs, it would be useful to understand the factors that cause it. These factors themselves are from a wide variety of individual disciplines within sports science; this section aims to identify the most pertinent of them, with a brief discussion regarding their effect on inter-subject variation to exercise. An earlier review by Mann et al. (2014) introduced some of these elements in the context of explaining inter-individual response to a standardised training programme, with subsequent reviews exploring more individual factors in greater depth (Camera 2018; Roberts et al., 2018). This section aims to build on this earlier work, as well as add some recent findings, particularly in the field of epigenetics. Once these factors have been identified, a series of models are created to examine the interaction between all these factors, increasing in complexity as the chapter progresses. Finally, ways to potentially harness and utilise this information using the new technology of genetic testing in order to improve exercise response within a population, with particular interest paid to elite athletes, are discussed.

2. Inter-subject variation in response to training

Typically, researchers are interested in understanding the mean response to an intervention in order to determine its overall efficacy. For example, when determining the effectiveness of resistance training in enhancing one-repetition maximum strength (1RM), subjects will undertake a pre- and post-training intervention 1RM test, with the average improvements reported. As an illustration, Hubal and colleagues (2005) recruited 585 previously untrained subjects to undergo a 12-week resistance training programme, with the mean 1RM improvement reported as 54%. Similarly, in randomised controlled trials, the mean pre-post change in the intervention group is compared to the mean pre-post change in the control group, and the effectiveness of the intervention determined. However, whilst sports coaches have long noticed that there is variation in how their athletes respond to a given training stimulus, it is only

relatively recently that interest in both quantifying and understanding this individual variation through structured research has developed (Bouchard 2012).

2.1 Inter-individual variation following aerobic training

The initial studies exploring this individual response from the perspective of aerobic training were published in the mid-1980s. The first, published in 1984, recruited ten monozygotic twin pairs to a 20-week endurance training programme, with pre- and post-intervention measures of maximal aerobic power (MAP), ventilatory aerobic (VAT) and anaerobic (VANT) thresholds determined. Whilst training enhanced post-training measures *on average* by between 12% (MAP) and 20% (VAT), there was considerable variation; for MAP, the magnitude of improvements ranged from 0-41% (Prud'Homme et al., 1984), for example. Subsequent studies replicated these initial findings (Despres et al., 1984; Simoneau et al., 1986), leading to the development of the large-scale HERITAGE (HEalth, RIsk factors, exercise Training And GEnetics) family study. Here, 720 subjects underwent a 20-week aerobic training programme, and undertook a battery of pre- and post-intervention tests (Bouchard & Rankinen, 2001). Again, the results showed significant individual variation; whilst the mean improvement in maximal aerobic capacity (VO_{2max}) was 384 mL O_2, some subjects saw an improvement of over 1000 mL O_2, and others a reduction in VO_{2max}. Similarly, whilst the mean improvement in heart rate (HR) at a workload of 50W was 11bpm, some subjects demonstrated improvements of greater than 40bpm, whilst a small number markedly worsened (Bouchard & Rankinen, 2001).

2.2 Inter-individual variation following resistance training

Following the initial interest in quantifying and exploring the inter-individual response to aerobic training, an increasing number of studies have explored the individual response to a resistance training programme. Perhaps the most famous of these was carried out by Hubal and colleagues (2005), who subjected 585 previously untrained males and females to an identical 12-week resistance training programme, comprised of three sessions per week. Whilst, on average, subjects improved their 1RM by 54%, and their muscle cross sectional area (CSA) by 19%, large inter-individual variations in these measurements were reported, with changes in CSA ranging from -2% to +59%, and changes in 1RM ranging from 0% to +250%. Bamman et al. (2007) recruited 66 untrained males and females to a 16-week resistance training programme, comprised of three weekly sessions. Using a cluster analysis, they stratified subjects into extreme, modest and non-responder groups; participants in the extreme group increased muscle CSA twice as much as those in the modest group. An analysis of three previous studies (Verdijk et al., 2009; Tieland et al., 2012; Leenders et al., 2013) by Churchward-Venne and colleagues (2015), comprised of training programmes lasting from 12-24 weeks, again reported significant heterogeneity in lean body mass gains (mean of +0.9kg, range -3.3 to +5.4kg) at the twelve week point, with concurrent large variations in improvements in 1RM (leg press; mean 33kg; range -36 to +87kg). Ahtiainen et al. (2016) reported training data on 287 male and female participants who had undertaken supervised resistance training programmes of 20-24 weeks duration. On average there was a significant increase in leg press 1RM of 21%, although again there was individual variation within that score, ranging from -8% to +60%. Similar results were reported for thigh muscle hypertrophy, with an average

increase of 4.8%, and an individual variation range of -10.6% to +30%. Finally, following 9 weeks of resistance training, Erksine and colleagues (2010) reported increases in 1RM ranging from 18 to 113%, increases in quadriceps muscle volume of 0-16%, and increases in MVC from -1% to +52%.

2.3 Other reported inter-individual variations

Other studies have reported large variations in response to high intensity interval training (Astorino & Schubert, 2014), fat loss and body composition (Barbeau et al., 1999; Barwell et al., 2009), other health-related aspects such as insulin sensitivity, blood pressure, and cholesterol levels (Bouchard & Rankinen, 2001), and even response to ergogenic aids such as caffeine (Jenkins et al., 2008). As a result, it is clear that inter-individual variation in response to a stimulus, including exercise, is a well-established phenomenon within the scientific literature.

2.4 Exercise response: modality specific?

One potential area for further exploration is whether this observed non-response is modality specific. Whilst the vast majority of studies reporting exercise non-response focus on a specific training modality, such as aerobic training (Prud'Homme et al., 1984; Bouchard & Rankinen, 2001) or strength training (Hubal et al., 2005; Erksine et al., 2010), a small number of studies examine exercise non-response across multiple modalities. Karavirta et al. (2011) randomised 175 male and female participants into four groups; endurance training only, strength training only, concurrent strength and endurance training, and a control group. All groups showed a large range in exercise response, with improvements in peak aerobic capacity (VO_{2peak}) ranging from -10 to +60% in the endurance trained group, and MVC improvements ranging from -15 to +60% in the strength trained group. But it is the strength and endurance trained group where the crucial data lies; although some participants saw a negative training response in either VO_{2peak} or MVC, not a single subject saw a negative response in both. In addition, no participant was in the highest quintile of improvement for both VO_{2peak} and MVC.

Similarly, Hautala and colleagues (2005) placed 73 participants through both an endurance and resistance training intervention in a randomised cross-over design, with improvements in VO_{2peak} as the outcome. As expected, there was individual variation in VO_{2peak} improvements from both the aerobic endurance (mean +8%, range -5 to +22%) and resistance training (mean +4%, range -8 to +16%) interventions, such that some participants did not improve with a given training modality. Interestingly, however, participants exhibiting the lowest magnitude of VO_{2peak} response following the aerobic training intervention exhibited a positive VO_{2peak} response following the resistance training intervention, lending credence to the possibility that changing exercise modality may eliminate, or at least reduce, exercise non-response. One potential limitation of this study is that each training intervention lasted only two weeks, a duration shorter than most training studies. Accordingly, it is possible that identified non-responders might have shown increased responses if the intervention period was over more standard timeframes, such as 6-8 weeks.

Finally, Bonafiglia and colleagues (2016) reported that, whilst there were non-responders in terms of VO_{2peak}, lactate threshold, and HR improvements following either endurance or sprint interval training, no subject was a non-responder to both exercise modalities, and very few were non-responders across all three measures for a single exercise intervention. As a result, it appears that non-response may be modality-specific; such a standpoint is supported by Booth & Laye (2010), whom stated that, with the thousands of biochemical adaptations to exercise, as well as the multitude of different training modalities, it seems incredibly unlikely that there are individuals who see no improvement at all following exercise. This is not necessarily a consensus, however, with Timmons (2011) writing that, whilst the inter-correlation in non-response to exercise between exercise modalities is low, it is not zero. Additionally, Bouchard et al. (2012) reported that across a number of exercise intervention trials, approximately 7% of participants experienced an adverse response to two or more markers of cardio-metabolic health. What is not clear is whether these adverse or non-responses would disappear with a different training modality or dose (Scharhag-Rosenberger et al., 2010). As exercise adaptation occurs through a number of separate pathways specific to the exercise modality, the potential lack of global non-responders also suggests the driver of individual differences in exercise response could be down to variation within these pathways.

3. Potential mechanisms driving the individual response

Having identified the potential for significant differences to exist between participants which determine the magnitude of response to exercise or other stimuli, the next step is to identify and discuss what might be driving these differences. This section will outline some of the main proposed mechanisms thought to underpin inter-individual variation in response to exercise.

3.1 Genetics

Contained within all nucleated cells are 23 chromosomes, which contain the genetic code in the form of deoxyribonucleic acid, commonly termed DNA. Alongside this, humans also have a small amount of genetic material contained within mitochondria, termed mitochondrial DNA (mtDNA). Together, the totality of these two aspects is termed the human genome. Within their chromosomes, humans have two complimentary strands of DNA; one inherited from their father, and one from their mother. DNA is comprised of base nucleotides, of which there are four; adenine (A), cytosine (C), guanine (G), and thymine (T). The order and number of these bases determines the protein(s) which can be produced from a particular gene. Three base nucleotides are required to produce a single amino acid, and these amino acids combine to create the proteins that drive of all the functions required for life. However, there is variation between humans with regards to the presence or otherwise of a certain base nucleotide at a certain point in a gene. When this variation is comprised of a single nucleotide substitution, it is described as a Single Nucleotide Polymorphism, or SNP. Here, one base has been substituted for another, which can change the amino acid that is encoded for, which in turn can change the protein that is produced, which in turn can have an effect on human function and performance. When it comes to understanding how and why genetic variation affects variation in response to a stimulus, it is research into such SNPs that has provided the greatest insight.

Following the completion of the Human Genome Project in 2003, genetic analysis has become increasingly affordable, making research into the effects of genes on fitness and performance more feasible. The knowledge of genetic influences has progressed significantly in recent years, and, as a result, there has been a shift from the idea that all traits are determined by a single gene, which still holds true in select disease states such as Cystic Fibrosis (Riordan 1989) and Huntington's disease (Walker 2007), to more complex polygenic interactions. The "single gene as a magic bullet" philosophy has also been present in sport (Davids & Baker 2007), with some coaches believing that single genes are responsible for athletic performance. However, no single gene has been discovered. Instead, it seems reasonable to assume that elite athletes are elite due to the possession of a number of alleles favourable for performance (Ruiz et al., 2009; Santiago et al., 2010). Even when an unrealistically low number of polymorphisms are examined, it is incredibly unlikely that one person possesses the perfect genetic profile for elite performance (Hughes et al., 2011; Williams & Folland 1998).

All traits, therefore, exist on a spectrum; from single gene traits, such as the Cystic Fibrosis phenotype at one end, to complex polygenic traits such as injury at the other. Whilst it might be thought that complex traits can never be fully understood in terms of their genetic component, recent research has identified candidate genes associated with complex traits such as intelligence (Davies et al., 2011), educational attainment (Rietveld et al. 2013), height (Silventoinen et al., 2003), and even chances of being an elite athlete (de Moor et al., 2007). Of course, these complex traits are also dependent on non-genetic factors, but there is a genetic component within them. Returning to exercise adaptation, it is now understood that the heritable component differs from trait to trait; for example, the results of HERITAGE suggest that approximately 50% of heterogeneity of improvement in VO_{2max} following aerobic training is determined by heritable factors (Bouchard et al., 1998), whilst variation in muscle fibre type is reported to be heritable in a range from approximately 45% to 99.5% (Komi et al., 1977; Simoneau & Bouchard, 1995). A recent meta-analysis reported that 52% of the variation in muscle strength phenotype is heritable (Zempo et al., 2017). Knowledge of these genes may allow manipulation of environmental factors such as volume, intensity, frequency and rest-periods to improve exercise response. Indeed, recent research has begun to argue whether true non-responders to specific exercise modalities exist; this is explored in greater detail in Chapter 3. Ross and colleagues (2015) conducted a study with three different aerobic exercise intensities. In the lowest intensity training group, almost 40% of participants were classified as non-responders. However, in the moderate intensity group, this number halved, and in the highest intensity group there were no non-responders to exercise. It could well be that response to exercise is linked to exercise intensity, although nevertheless there still appears to be large variation in terms of what the ideal intensity is for each person (Bonafiglia et al., 2016).

Given that the vast majority of traits are polygenic in nature, there is considerable debate on the "optimal" method for identifying genetic variants associated with a variety of traits, including exercise adaptation (Pitsiladis et al., 2013; Bouchard 2015). This is explored further in Chapter 4. Initially, the field of exercise genomics was built on twin studies, in which the phenotypes of monozygotic (MZ; identical) twins are compared to the phenotypes of dizygotic (DZ) twins. If a phenotype appears more concordant in MZ compared to DZ twin pairs, then it is likely strongly influenced by genetic variation. This approach was initially used to estimate the heritability of VO_{2max} (Williamson et al., 2017).

As genetic testing technology improved, researchers began to utilise single-SNP models of research, most often with case-control or candidate gene analysis. In this model of research, relative genotype frequencies for a SNP/gene of interest are compared between individuals with a phenotype of interest (cases) and controls. For example, variation in *ACTN3,* which influences relative type-II fibre types, was initially elucidated when comparing elite speed-power athletes with non-athletic controls (Yang et al., 2003). Common criticisms of the candidate gene approach are that it requires prior knowledge that a specific SNP may have an effect, and that many studies utilising this methodology are underpowered (Bouchard 2015); as a result, replications of SNPs elucidated via this method are often lacking (Ahmetov et al., 2010; Ahmetov et al., 2016).

Due to these criticisms, there has been a move towards Genome-Wide Association Studies (GWAS). Here, a large number of SNPs (>100,000) are compared between cases and controls, allowing for the discovery of novel SNPs. Due to the large number of comparisons that occur, the typical threshold for statistical significance within GWAS is $p<5 \times 10^{-8}$. As many SNPs likely have small effect sizes on the trait of interest, this requires exceptionally large numbers of subjects to be recruited to these studies (Mattsson et al., 2016) – problematic when dealing with elite athletes, who by definition are few in number. Additionally, some authors (Manolio et al., 2009; Yang et al., 2010) argue that this threshold is too high, and is a common cause of the "missing heritability problem", detailed further in Chapter 8 (Dudbridge 2013).

As an illustration of the potential issues of the different approaches, many SNPs identified through candidate gene studies as having an effect on a specific phenotype are often not replicated in subsequent GWAS. Returning again to *ACTN3*, candidate gene studies show a clear, and well replicated, role for the R allele in elite speed-power performance, with this outcome demonstrated at meta-analysis level (Ma et al., 2013; Tharabenjasin et al., 2019). However, GWAS of elite sprint athletes (Wang et al., 2014) or sprint speed in soccer players (Pickering et al., 2019a) have not identified *ACTN3* as being associated with either trait. This represents a current impasse; are the previous candidate gene studies wrong, or is the threshold for discovery in GWAS too high? The right answer is likely a mixture of the two, and recent studies utilising lower thresholds for discovery from GWAS appear to hold enhanced predictive and explanatory power (Shi et al., 2016; Boyle 2017; Khera et al., 2018). As this field progresses, further methodological innovations are expected.

3.1.1 Gene polymorphisms & exercise adaptation

A recent review (Ahmetov et al., 2016) reported that at least 155 genetic markers have been associated with elite athlete status, with approximately 10% of these replicated in at least three studies; more genes still are implicated with exercise adaptation (Bray et al., 2009). Research in elite athletes is a good starting point in the search for candidate genes driving exercise response, as this population represent a highly specialised cohort. For example, elite sprinters are likely very good at sprinting because they possess alleles that predispose them to adapt favourably to speed-power training. One such gene that may play a role here is *ACTN3*, which creates α-actinin-3, a protein that forms part of the Z-line in muscle fibres. A SNP within *ACTN3*, known as R577X, arises from a C → T substitution, resulting in

the formation a premature stop codon (X) in the place of arginine (R). Approximately 18% of individuals are homozygous for the X allele (North et al., 1999), causing them to be deficient in α-actinin-3. Whilst this lack of an R allele is not associated with any disease state, it does mean that XX genotypes tend to have a lower percentage of type-IIx muscle fibres (Vincent et al. 2007). Yang and colleagues (2003) examined the impact of this polymorphism in elite sport, comparing *ACTN3* genotypes between three groups; elite power athletes, elite endurance athletes, and non-athletic controls. Within the control subjects, the XX genotype had a prevalence of 18%; however, it did not occur in any of the power Olympians, indicating that the XX genotype is unfavourable for elite power performance. Conversely, the XX genotype was present in approximately 35% of endurance Olympians, suggesting it potentially has a beneficial effect on endurance performance. Subsequent research has confirmed the association between the R allele and power performance (Ma et al., 2013), although the link between the X allele and endurance is less clear (Papadimitriou et al., 2018). Other genetic variants found to affect athletic performance with replication include *ACE* I/D (Collins et al., 2004; Gayagay et al., 1998; Nazarov et al., 2001), *PPARGC1A* Gly482Ser (Eynon et al., 2009a; Lucia et al., 2005; Maciejewska et al., 2012), *GABPB1* (rs7181866) (Eynon et al., 2009b; Maciejewska-Karlowska et al., 2012), *BDKRB2* +9/-9 (Saunders et al., 2006; Williams et al., 2004), and *HIF1A* Pro582Ser (Doring et al., 2010a; Gabbasov et al., 2013), to name but a few.

After identifying a relevant polymorphism, the next step is to apply this knowledge within studies examining the individual training response. Returning to *ACTN3*, Delmonico et al. (2007) examined changes in knee extensor power following 10-weeks of strength training in older adults, discovering that RR genotypes showed greater improvements in peak power than XX genotypes. Similarly, Pereira et al. (2013) examined training response following a 12-week resistance training programme in elderly women. All *ACTN3* genotypes demonstrated significant improvements in tests of strength and power following training, but these improvements were greatest in R allele carriers. The mechanisms driving these individual changes are not yet fully understood. Norman et al. (2014) reported that exercise-induced increases in mTOR and p70S6k, stimulators of skeletal muscle hypertrophy, were greater in R allele carriers than XX genotypes following high intensity exercise. Ahmetov and colleagues (2014a) reported that testosterone levels were significantly higher in RR genotypes compared to XX genotypes in a group of both male and female Russian athletes. R allele carriers also tend to have a higher percentage of type-II muscle fibres (Ahmetov et al., 2012), which might allow for greater amounts of hypertrophy following resistance training (Campos et al., 2002; Fry 2004). These aspects go some way to explaining the differences in training responsiveness between *ACTN3* genotypes. Once again, similar research has shown a modifying effect of other polymorphisms on training response, including *ACE* I/D (Cam et al., 2007; Folland et al., 2000; Giaccaglia et al., 2008; Moraes et al., 2018) and *PPARGC1A* Gly482Ser (Ring-Dimitriou et al., 2014; Stefan et al., 2007; Steinbacher et al., 2015).

Research also indicates that genes can influence other traits affecting exercise performance. These include injury risk (Brazier et al., 2019)—where SNPs in genes such as *COL1A1* (Posthumus et al, 2009a, 2009b) and *COL5A1* (Mokone et al., 2006; Posthumus et al., 2009c; September at al., 2009) can influence the risk of tendon and ligament injury—and recovery speed, where SNPs in *IL6* and *TNFA* have the potential to influence post-exercise inflammation (Yamin et al., 2008).

Genetic research is still in its infancy and is rapidly developing. The future for this field is promising, with many more polymorphisms affecting exercise adaptation remaining to be elucidated. As the cost of complete genome sequencing drops, there will be an increased ability to conduct Genome Wide Association Studies (GWAS)—generally considered the gold standard (Pitsiladis et al., 2013)—in large cohorts, along with replications to remove false positives (spurious positive genetic associations); this should further enhance the knowledge in this area.

3.2 Environmental factors

If heritable factors are responsible for a part of exercise adaptation, the obvious question to ask is – what is responsible for the other part? These non-genetic factors are often termed "environmental" which are defined for the purpose of this literature review as non-genetic factors. This section divides them into four groups; individual history, programme design, psycho-emotional factors (including stress and prior beliefs), and nutrition. These non-genetic factors can be both acute, affecting a single or small number of consecutive sessions—such as a single poor night's sleep—or chronic, affecting response to the training programme as a whole; long-term sleep deprivation, for example. There are doubtless many more non-genetic factors that can influence exercise response to varying extents; this section focuses on those with the largest effects.

3.2.1 Individual history

A phenotype is the observable expression of an individual's genotype (Wojczynski & Tiwari 2008), which is impacted by that person's environment (Winawer 2006). In terms of exercise response, individuals can be viewed as having a highly-, normal-, or under-adaptive phenotype, influenced by their genotype (see 3.1), but also environmental variables. One such variable is that of baseline fitness, which can impact recovery from exercise (Hagberg et al., 1980; Short & Sedlock, 1997; Tomlin & Wenger, 2001). Another is training history, with previously trained individuals showing differences in adaptive mechanisms post-exercise when compared to beginners (Coffey et al., 2006); this effect can also be modified by subject age (Kosek et al., 2006). These differences might, however, be lost with detraining (Lindholm et al., 2016). When looking at dietary interventions, diet history can also have a modifying impact on responsiveness to the diet, with previous weight loss attempts potentially making future weight loss harder (Higginson & McNamara 2016). Finally, higher habitual physical activity can increase the response to endurance training (Hautala et al., 2012). Within HERITAGE, environmental correlates of VO_{2max} improvements following training included baseline VO_{2max}, age, gender, weight, ethnicity, and whether or not the subject achieved the target workload (Sarzynski et al., 2016). Baseline phenotypes themselves appear to influence separate traits to different extents; comprising a smaller portion of VO_{2max} improvements following exercise (11-16%) and a larger portion of blood pressure response following exercise (21-47%) (Mann et al., 2014).

3.2.2 Programme design

Training programme design (exercise selection, frequency, duration, intensity, recovery times, repetition and set ranges, etc.) can also influence the magnitude of adaptation to training (Campos et al., 2002; Contreras et al., 2016; Fry 2004; Rossi et al., 2016; Schoenfeld et al., 2016a, Schoenfeld et al., 2016b; Wilson et al., 1993), as can time of day of training (Ammar et al., 2016; Facer-Childs & Brandstaetter, 2015), such that two people with an identical genotype doing different training programmes would see a difference in phenotype. Indeed, Sisson and colleagues (2009) found that total exercise volume was a factor in the number of non-responders to exercise; by increasing volume threefold, the number of non-responders to an aerobic training intervention was reduced from 45% to 19% 19%, suggesting that environmental influences can perhaps over-ride the genetic pre-disposition to exercise non-response.

3.2.3. Psycho-emotional factors

In recent years, attention has turned to how non-physical, psycho-emotional factors can influence exercise performance and adaptation. Initially, this attention was focused on fatigue, with both the Central Governor (Noakes, 2012) and psychobiological (Smirmaul et al., 2013) models proposed to explain the relationship between the brain and fatigue. Since this initial foray into multi-disciplinary approaches to explain exercise behaviour, evidence illustrating how inter-individual and transient psycho-emotional considerations influence individual responses to exercise adaptation has continued to grow. The influence of psycho-emotional factors on numerous dimensions of performance has recently been illustrated within athletic preparation contexts (Kiely 2016; Mann et al., 2016; Stults-Kolehmainen et al., 2016). Similarly, the mechanisms underpinning how psycho-emotional states mediated biological adaptations have been extensively outlined within the wider psycho-biological (Ganzel, 2010) and training specific (Kiely, 2018) literatures.

Existing evidence illustrates that the sense of threat imposed by applied stressors—such as exercise-induced stimuli—are evaluated, by the brain's emotional centres, against the organisms perceived capacity to cope with that stress (Ganzel et al., 2010). The direction and magnitude of the consequent changes in the neuro-chemical environment (i.e., the stress response) is subsequently heavily modulated by the emotional valence attached to that stressor, as the organism strives to appropriately prepare for the forecasted challenge. These neurochemical alterations subsequently drive down-stream biochemical changes; thereby altering the biochemical backdrop upon which straining stimuli are overlaid and, subsequently, modulate the adaptive response to exercise (Viru & Viru, 2004; Kraemer & Ratamess, 2005). From this, it is apparent that the size of the response to a stressor is not merely dependent on the magnitude of the stressor itself, but also on the emotional evaluation of the perceived threat posed by that stressor. In effect, emotional valence drives changes in the neurobiological chemical milieu, which in turn influences physiological adaptation.

The emotional response itself is complex and is perhaps best summarised by Ganzel and colleagues (2010). In their model, the authors describe some of the factors that mediate this emotional

response. These include prior context, such as previous traumatic experiences, evolved coping behaviours, and general health. This prior context then interacts with the current state of the organism, both in terms of emotional capacity (influencing prior mental health, which in turn influences the acute emotional response to a stressor) and, via the chemical changes that drive subsequent physical responses, physical changes that accompany chronic stress, such as increased cortisol (which influences prior physical health, itself a modifier to the acute response to a stressor). These factors combine to shape the perception of threat posed by any given stressor, and this perception of threat in turn shapes the specific set of neurobiological responses launched to combat the forecasted challenge.

Consequently, it is worth noting that every stressor, including exercise, exerts a neurological, biological, psychological, cognitive and emotional load. The magnitude and direction of this load is highly context- and individual-specific, and inextricably modulated by non-physical factors; meaning that, at the individual level, adaptations in response to exercise will inevitably be modulated by a host of non-physical mediators, thereby further customising inter-individual adaptive responsiveness.

3.2.3.1 Factors affecting psycho-emotional response

This response to a stressor is altered by both environmental and genetic factors. Focusing on the environment, one such factor is lack of sleep, which can impact exercise recovery (Dattilo et al., 2011; Leeder et al., 2012a), and promote the release of stress hormones (Dattilo et al., 2012). This can lead to a reduction in aerobic endurance (Azboy & Kaygisz, 2009) and strength (Souissi et al., 2008) performance, and also increase the inflammatory response (Heffner et al., 2012)—potentially altering training performance and hence adaptation. A lack of sleep, for example, increases vulnerability to a mild stressor (Minkel et al., 2012) and alters psychological coping mechanisms (Akersted et al., 2012). The interpretation of stress is also modified by heritable factors; polymorphisms in genes such as *COMT* (Clark et al., 2013)*, BDNF* (Clasen et al., 2011; Colzato et al., 2011) and *5HTTLPR* (Clasen et al., 2011) have been shown to impact both the acute and chronic stress response, which in turn can modify exercise adaptation (Petito et al, 2016) and performance (Sanhueza et al., 2016). The microbiome, which is influenced by both environmental and genetic factors, can also alter the stress response in athletes (Clark & Mach, 2016). Finally, epigenetic modifications (see section 3.4) will affect the stress interpretation pathways (Nestler 2012), providing an explanatory framework depicting how childhood trauma can influence adult stress behaviours decades later (Heim & Binder, 2012).

The individual response to a stressor can have a sizeable impact on the adaptive mechanisms following exercise. Psychological stress can affect exercise adaptation by decreasing immunity and recovery (Clow & Hucklebridge, 2001), as well as increasing the risk of injury (Mann et al., 2016). Additionally, baseline stress has been correlated with VO_{2max} improvements (Ruuska et al., 2012). Given that the stress response is partially hormone led (Huang et al., 2006), and these hormonal changes can be fast-acting (Cook & Crewther, 2012), the stress state of the subject at the time of exercise has the potential to modify the adaptive response both acutely and chronically (Main et al., 2010). To illustrate this, Bartholomew and colleagues (2008) reported that after 12 weeks of resistance training, participants with lower levels of self-reported lifestyle stress had greater increases in both bench press and squat

strength compared to those participants with a high stress score. Similarly, a subject who has just argued with a spouse and has long-term financial worries is likely to have less resources available to mount an optimally healthy adaptive response than a subject who is content (Kiely, 2016).

The acute psycho-emotional response to a training session could also possibly cause variation in work rate within that session, which may itself determine some of the inter-subject variation in response to exercise (Sarzynski et al., 2016). This work-rate is likely comprised of many factors, including residual fatigue, but also via psychological factors which can influence within-session work via the psychobiological model (Smirmaul et al., 2013). Individual variation in perception of work rate can lead to changes in exercise performance (Marcora & Staiano, 2010), and this perception of work rate is influenced by a myriad of factors (for review, see Noakes 2012). The perception of effort also has a heritable component, which explains 35% of the variance in rating of perceived exertion (RPE) between participants (Schutte et al., 2017). Furthermore, acute alterations in hormone levels may influence individual motivation to train and perform physical work (Crewther et al., 2016; Crewther et al., 2018). Variation in these all of these factors could alter within-session work rate, in turn modifying exercise adaptation.

Finally, expected and previously-held beliefs can impact the emotional evaluation of a stressor, again potentially modifying training performance and adaptation. An ever-increasing body of literature has illustrated that a subject's prior beliefs can modify how they perform within a session, including belief that they have consumed caffeine (Saunders et al., 2016), steroids (Ariel & Saville, 1972; Maganaris et al., 2000), sodium bicarbonate (McClung & Collins, 2007), and doping agents (Ross et al., 2015) within blinded experiments. Returning briefly to sleep, "placebo sleep"—whereby individuals are informed they've slept for longer than they actually have—can improve cognitive function (Draganich & Erdal, 2014), again illustrating the power of belief. The nocebo effect can also influence exercise outcomes (Pollo et al., 2012). Given that expected beliefs can alter effort within a training session, whether a subject believes a specific exercise or training programme is either positive or effective can alter the outcome of exercise- and nutritional-intervention trials (Beedie & Foad, 2009; Mothes et al., 2016). Interestingly, some research seems to suggest that certain genotypes are more sensitive to expectancy, placebo and nocebo effects (Hall et al., 2015), once again illustrating the consistent influence of genetics on environmental factors.

3.2.4. Nutrition

An additional factor that can influence exercise adaptation is that of nutrition. Macronutrient intake impacts both exercise performance (Bergström et al., 1967) and exercise adaptation (Bartlett et al., 2014; Hammond et al., 2016). The same is true for micronutrients, with serum vitamin D levels associated with muscle power and force, both acutely (Ward et al., 2008), and in response to a training programme (Close et al., 2013). Recently, attention has focused on individual variation in the gut microbiota, which again can modify the response to exercise (Mach & Fuster-Botella, 2016), including modifying post-exercise recovery and mood states (Clark & Mach, 2016). Finally, long-term high dose antioxidant use can potentially blunt the adaptive response to exercise (Draeger et al., 2014; Ristow et al.,

2009)—although these findings are not unequivocal (Yfanti et al., 2010)—leading to the possibility that differences in dietary composition could cause some of the inter-subject variation seen in response to exercise. Other nutritional factors can modify the acute physiological stress expected following training; these include short-term macronutrient intake (Hawley et al., 2011), antioxidant intake (Braakhuis & Hopkins, 2015)—which can be both positive or negative depending on the nutrient and dose (Braakhuis et al., 2014)—and use of medications such as non-steroidal anti-inflammatory drugs (NSAIDS) (Mackey et al., 2007; Mikkelsen et al., 2009; Trappe et al., 2002).

These nutritional factors are also influenced by genetic aspects. The microbiome, for example, is influenced by host genetics (Bonder et al., 2016). Returning to vitamin D, polymorphisms in a variety of genes, including *VDR,* can influence muscle strength (Grundberg et al., 2004), which will in turn influence response to training. *VDR* can also influence a person's vitamin D requirements (Graafmans et al., 1997). Close and colleagues (2013) demonstrated that vitamin D supplementation enhances improvements to a strength training programme, which raises the question - do non-responders to strength training not respond because of genetic factors, or is their response blunted due to vitamin D insufficiency (which in turn can be influenced by SNPs)? Given that nutrition can also impact gene signalling post-exercise (Arkinstall et al., 2004; Churchley et al., 2007), it's easy to see how both genes and environment combine and interact to create the phenotype.

Finally, the use of ergogenic aids can also affect the performance level within an individual training session, which in turn can modify the overall adaptation that accumulates over time. One such aid is caffeine, which has a clear and replicated ergogenic effect on exercise performance (Astorino & Robertson, 2010; Doherty & Smith, 2004; Graham, 2001). Caffeine is examined in greater detail in Chapter 5. Another is creatine, which can affect intra-session recovery (Mujika & Padilla, 1997), potentially allowing for a greater volume of high intensity efforts to be completed.

3.3. Summarising gene-environment interactions

Having discussed the different genetic and environmental aspects that can affect exercise response, it is worthwhile summarising these within a model. Figure 1 shows the typical gene and environment model, where genetic and environmental factors are kept separate, and combine in an additive manner to determine the post-exercise adaptation phenotype. As a simplified example, two individuals homozygous for the R allele of *ACTN3* will have different phenotypes based on environmental factors. If subject A undertakes high-load resistance training, they will likely see good levels of muscle hypertrophy. If subject B is sedentary, then they won't see hypertrophy, no matter how positive their genotype is.

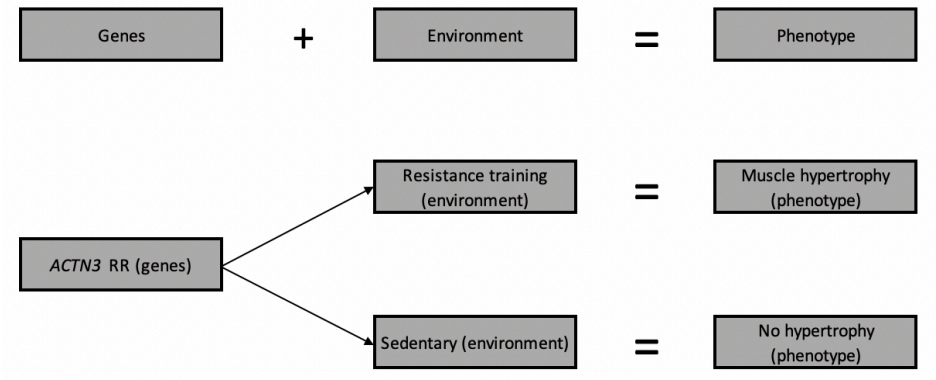

Figure 1 – The Gene-Environment Model with *ACTN3* example.

However, as explored in all the sections within 3.2, it is clear that there are a variety of environmental factors that can affect training response. These factors have a complex relationship with genetic factors; they can affect genetic expression, but are also affected themselves by SNPs within specific genes. This allows the formation of a more complex model, as per figure 2, which illustrates the increasing complexity.

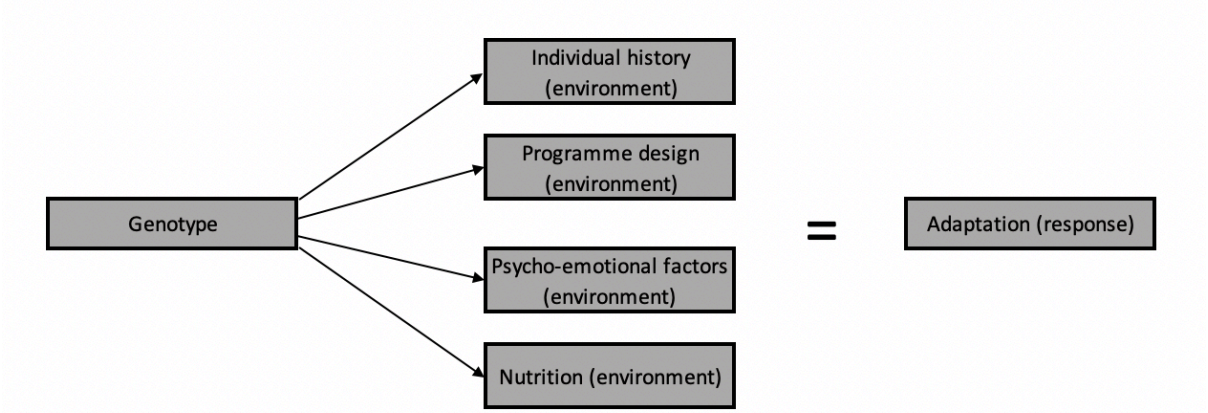

Figure 2 – Multi-environmental interactions with genotype. A more complex model illustrating the relationship between various environmental and genetic factors to create the outcome, in this case exercise adaptation. Further complexity could be added to this model by showing the inter-relationship between the environmental factors; nutrition can affect psychological factors, for example.

3.4 Epigenetics

Having introduced genetics and environment, two aspects that are typically thought to combine to create the phenotype, the next area to explore is epigenetics, the process by which environment can influence genetic expression. Epigenetics can be defined as changes in gene function that occur without a change in the nucleotide sequence (Ling & Groop, 2009). These changes have the potential to be heritable—although this is controversial (Horsthemke, 2018)—but also changeable over the course of time within an individual (Moran & Pitsiladis, 2016). The three main epigenetic mechanisms that have been elucidated thus far are DNA methylation, histone modifications, and non-coding mRNA (Moran &

Pitsiladis, 2016); all act as a way for the environment, through factors discussed in section 3.2, to modify genetic expression.

3.4.1.DNA methylation

The most extensively studied epigenetic mechanism, DNA methylation occurs through the addition of a methyl group to a cytosine base (Ehlert et al. 2013), which in turn makes that section of DNA less accessible for translation (Rottach et al., 2009). This can be positive or negative depending on whether expression of that gene is desired; methylation of oncogenes (Ehrlich 2002) and obesity-risk genes (Nitert et al., 2012) is likely positive. In contrast, methylation of tumor suppressor genes (Ehrlich 2002), and those that drive exercise adaptation (Nitert et al., 2012) is less ideal. Fortunately, the same stimulus can lead to both an increase and decrease in methylation in different genes (Voisin et al., 2015). As an example, six-months of aerobic exercise lead to a decrease in methylation (hypomethylation) in muscle genes (Nitert et al., 2012), promoting adaptation, and an increase in methylation (hypermethylation) in adipose tissue genes (Ronn et al., 2013), potentially stimulating weight loss. Similarly, *PPARGC1A,* a gene that drives mitochondrial biogenesis following exercise (Eynon et al., 2011), showed an increase in methylation following nine-days of bed rest, and a decrease in methylation following four-weeks of re-training (Alibegovic et al., 2010).

DNA methylation is modifiable within an individual; the DNA methylation profiles of obese patients become more like those of lean subjects' following a weight-loss intervention, for example (van Dijk et al., 2015). The levels of DNA methylation in response to the same stimulus may also change over time, with higher levels seen in elderly subjects following exercise—potentially due to the greater accumulation of aberrant methylation in these participants that needs correcting with exercise (Brown 2015). Of the three types of epigenetic modification detailed here, DNA methylation is perhaps the most stable (Ehlert et al., 2013), with early life experiences, even those pre-birth, potentially exerting a long-term effect on gene expression (Champagne, 2010). From an exercise perspective, this was recently explored by Seaborne and colleagues (2018). Here, the authors placed eight previously untrained males through a seven-week resistance training programme, followed by a seven-week washout phase, before undertaking a further seven-week loading period. After completion of the initial training programme, a number of epigenetic modifications occurred, including both hypo- and hypermethylation. These methylation markers were largely retained during the washout period, and then enhanced during the subsequent loading period, leading the authors to suggest that skeletal muscle has an "epigenetic memory", potentially making adaptation to a subsequent training load more efficient.

Finally, DNA methylation patterns may even be passed from generation to generation (Voisin et al., 2015), leading to the interesting possibility that methylation markers affecting elite athlete status and fitness levels may be partially inherited, although a more comprehensive body of work is required to explore this hypothesis (Horsthemke 2018).

3.4.2 Histone modifications

DNA is coiled around histone proteins, giving it a specific shape. The epigenetic variation caused by histone modifications occur via acetylation of this structural histone, which changes its shape. This makes the specific section of DNA comparatively more available for translation, which in turn makes the expression of these genes easier (McGee et al., 2009). The process of acetylation of histones is controlled by a histone acetyl-transferase (HAT), whilst histone deacetylase (HDAC) can remove the acetyl group, reducing translation at that point (McKinsey et al., 2001). In mice, it has been shown that the presence of a particular HDAC (HDAC5) can reduce the adaptations expected following exercise (Potthoff et al., 2007), showing how histone modifications might affect exercise response. In humans, HDAC5 levels are lower following training, confirming that these proteins play a role in exercise adaptation, although at present it's not exactly clear what causes individual differences in HDAC5 levels (McGee et al., 2009). Histone modifications represent the most transient of the epigenetic changes, and are constantly in a state of flux (Ehlert et al., 2013).

3.4.3 Non-coding RNA

RNA is typically used by the body as messenger RNA (mRNA) to pass information from DNA to the ribosomes, where protein synthesis occurs. However, the vast majority of RNA found within the body is non-coding (Bernstein et al., 2012); instead, this RNA might regulate genetic expression or catalyse chemical reactions. Regarding epigenetic translational alterations, of interest is micro RNA (miRNA), molecules which appear to exert control over mRNA, either by inhibiting translation or causing degradation before translation occurs (Guay et al., 2011). This indicates miRNA could regulate gene transcription post-exercise, affecting adaptation. In participants matched for diet, training history, age and body mass, a 12-week resistance training programme elicited adaptations of differing magnitude, in part mediated by specific miRNAs; levels of these miRNAs were correlated with greater adaptations, such as increases in strength (Davidsen et al., 2011), a finding which has been replicated (Hagstrom & Denham, 2018). miRNA has also been reported to play a role in aerobic exercise adaptation (Bye et al., 2013; Mooren et al., 2014; Timmons et al., 2010; Zhang et al., 2017). Similarly, there is disparity in the circulating levels of specific miRNAs between athletes primarily engaged in either strength or aerobic endurance training (Hecksteden et al., 2014). miRNAs may also reflect the level of fatigue of the athlete (Hecksteden et al., 2014), showing potential as a monitoring tool within elite sports programmes, although further research is required to enhance understanding in this area (Fernandez-Sanjurgo et al., 2018). It's not entirely clear at present which factors affect circulating levels of miRNA, making it difficult to harness this knowledge to improve performance, but the use of miRNA as a marker of exercise adaptation and load remains an area of increased interest from both a sporting and health perspective (Hecksteden et al., 2014; Polakovicova et al., 2016; de Gonzalo-Calvo & Thum, 2018; Ultimo et al., 2018).

At this point, an updated model, including the impact of epigenetics on gene-environment interactions, can be created, as seen in figure 3.

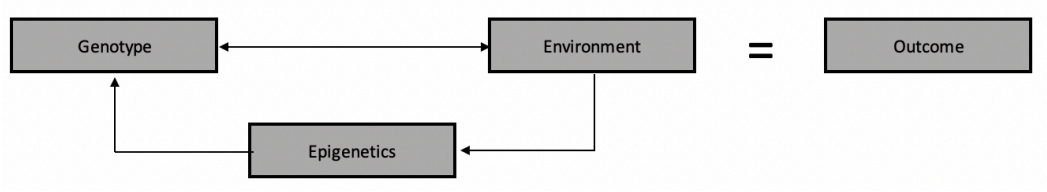

Figure 3 – A simple model of gene-environment interactions, with the addition of epigenetics. In this model, environmental factors have been grouped together for simplicity. Here, these environmental changes alter genetic expression, although as discussed in 3.4.4 and 3.4.5, this is a complex relationship.

3.4.4 Genetic influences on epigenetic modifications

So far, I have covered how epigenetic mechanisms allow for the environment to impact genetic expression, which is typically how epigenetics is viewed. However, genetic variation can also affect the efficiency of epigenetic modifications, allowing things to come full circle. This has been most well studied in terms of methylation, where a number of genes (Gertz et al., 2011), perhaps the most well-known of which is *MTHFR* (Friso et al., 2002), affect DNA methylation, and in turn can modify epigenetic modifications following exercise. Teruzzi et al. (2011) reported that elite athletes had a greater number of polymorphisms across several genes that affect methylation status, resulting in a genetic predisposition to hypomethylate. This lack of methylation potentially increases post-exercise muscle hypertrophy by increasing specific gene transcription. These findings were replicated by Zarebska et al. (2012), in which the authors speculated that there was an advantage in being a heterozygote of *MTHFR* A1298C. The proposed mechanism was that heterozygotes had decreased methylation of adaptive genes, which wasn't the case in AA homozygotes, but didn't exhibit increased homocysteine levels, associated with lower muscle strength (Kuo et al., 2007a), common in CC genotypes.

3.4.5 Environmental influences on epigenetic modifications

Along with genetics, environmental influences such as nutrition can alter epigenetic modifications; a high calorie diet appears to increase methylation of genes controlling metabolism, making metabolic dysfunction more likely, for example (Brøns et al., 2010). As discussed in 3.4.4, genes influence the efficiency of methylation, but also interact with environmental factors to control these changes, adding an extra layer of complexity. As an example, *MTHFR* encodes for an enzyme that coverts the folate derivate 5,10-methylenetetrahydrofolate (5,10MTHF) to 5-methylterahydrofolate (5MTHF), creating s-adenosylmethionine (SAM) – an agent for DNA methylation. Simply put, this pathway starts with folate, which is converted to intermediaries by MTHFR, with the availability of these intermediaries affecting methylation efficiency (Niculescu & Zeisel, 2002; Shelnutt et al., 2004). The two *MTHFR* SNPs, C677T and A1298C, influence the activity of the MTHFR enzyme. Focusing on C677T, T allele carriers typically have poorer conversion of folate, which in turn can influence methylation. Shelnutt and colleagues (2004) examined this in detail, placing participants on a low folate diet (≈115μg/d) for seven weeks, and then a high folate diet (≈400μg/d) for seven weeks. There was a trend

for decreased methylation within both groups with low folate intake; however, this trend was reversed during the high folate phase, and to a greater extent in TT genotypes.

Exercise is another environmental influence that can modify epigenetic changes through alterations in gene silencing and expression (Ntanasis-Stathopoulos et al., 2013), similar to that discussed in 3.4.1 and reviewed by Voisin et al. (2015). The homeostatic stress caused by exercise drives epigenetic modifications (Sanchis-Gomer et al., 2012), which in turn can lead to exercise adaptations by increasing translation and transcription of enzymes involved in adaptive mechanisms, such as AMPK and PGC-1a (Pareja-Galeano et al., 2014).

Environmental influences on epigenetic modification can play a role in determining an individual's phenotype; in individuals with the same genotype (monozygotic twins), differences in environment lead to different epigenetic changes occurring (Fraga et al., 2005), which can, for example, affect type-II diabetes risk, (Kapiro et al., 1992). Alongside nutrition and exercise, other environmental factors that can influence epigenetic modifications include psychological trauma (Yehuda, et al., 2005; Yehuda et al., 2009), which can potentially be passed down generations (Dias & Ressler, 2014), but also reversed (Weaver et al., 2005). Environmental toxins such as tobacco smoke, dietary polyphenols, alcohol and shift work can also all modify epigenetic regulation (Alegria-Torres et al., 2011).

4. A final model to explain the causes of inter-subject variation

As detailed in 3.1, genetic variation can clearly modify the magnitude of adaptation to exercise, both in single SNP/gene (e.g. *ACTN3*) and combined gene (e.g. HERITAGE) models. Section 3.2 examined non-genetic aspects that influence this response, including nutritional status (both chronic and acute) and training history. As an example, total daily protein intake impacts muscle protein synthesis following resistance training, and vitamin D status can modify performance (Close et al., 2013). These non-genetic factors are also affected by genetic factors; serum 25(OH)D levels both before and after supplementation are affected by specific SNPS (Didriksen et al., 2013). It is clear, therefore, that genetic and non-genetic factors are linked. The same is true for acute environmental factors, such as a stressor, which, as discussed in 3.2.3, might affect response to a single exercise bout. These acute factors will be influenced by genetic aspects, such as a SNP in *COMT* that can modulate stress response (Stein et al., 2006). They will also be influenced by environmental factors such as previous trauma (Nemeroff, 2004).

Section 3.4 discussed epigenetics, which is a mechanism through which environmental aspects can influence genetic expression; 3.4.4 and 3.4.5 explored how genetic and non-genetic factors also influenced epigenetic modifications, further illustrating the complex relationship between all factors, and requiring an update to the model proposed in figure 3. This culminates in a model of how all these factors interact to create a unique response for each individual in response to a stimulus. This response is not stable, as the component factors themselves can be highly variable over time; just because an individual saw a performance improvement after one training programme does not guarantee the same improvement

following the same programme once more (Robertson et al., 2010). This complex relationship is illustrated in figure 4.

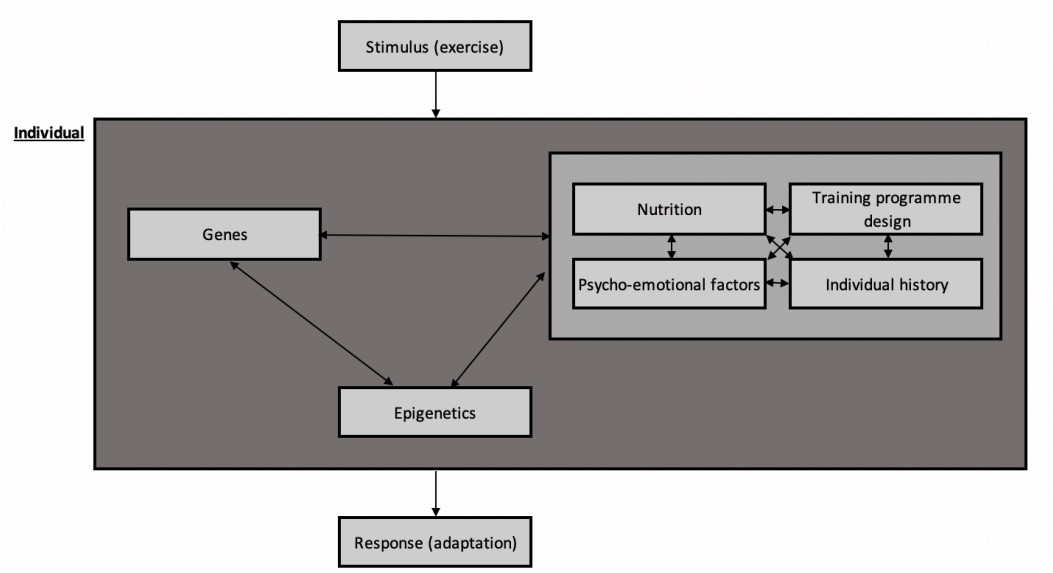

Figure 4 – A final model to explain inter-subject variation in exercise response. The complex interaction between genes, environment and epigenetics on response to a stimulus, in this case a training programme.

5. Harnessing this knowledge to improve performance

Having discussed the main aspects that affect individual adaptation to exercise, the next step is to attempt to make this information usable to athletes. Being able to compete at the highest level is a function of talent alongside optimal training – but how does an athlete know their training or use of an ergogenic aid is optimal? Typically, this requires trial and error, which is costly in terms of both time, and, if the trial is ineffective, performance. As athletes only have a window of a few years to be able to compete at their peak, time spent doing sub-optimal training can be very damaging. Being able to have more information on which to base decisions regarding training methodology would obviously be very attractive to everyone involved in sport. Currently, most testing carried out in athletes is of the phenotypic variety; VO_{2max} and vitamin D tests being two examples. This testing has use, as it provides a snapshot of where the athlete is at a given point in time; it can inform training requirements, but has minimal long-term predictive ability.

Given that a large proportion of inter-subject variation is down to genetic factors, testing for these factors through a genetic test might hold some promise. This could be single gene/SNP testing, or, more promisingly, large scale testing such as partial or whole genome sequencing. The cost of these tests has dropped in recent years (Hayden, 2014), making them much more accessible. This raises the potential for genetic tests to be used to inform training programme design, which may have some predictive ability (Jones et al., 2016; Monnerat-Cahli et al., 2016; Timmons et al., 2010). Whilst single gene models might give some insight into how humans respond to exercise (Delmonico et al., 2007; Kikuchi & Nakazato,

2015; He et al., 2018), adaptation to exercise is not determined by a single gene. Instead, groups of genes influence the various different cellular pathways (Camera et al., 2016) that control adaptation. By looking at just one gene, such as *ACTN3*, there is a risk of ignoring the effects of these other genes. One way to overcome this is to use a multi-gene model, comprised of an algorithmic approach that allows for the evaluation of a number of gene polymorphisms.

One method that has been used in this regard is that of the Total Genotype Score (TGS). This method has been used against retrospective data to improve identification of at-risk individuals for both cardiovascular disease (Thanassoulis et al., 2012), and type-II diabetes (Meigs et al., 2008). Within the sports and exercise world, TGS have so far been examined primarily as a potential tool for the discovery of elite athletes (Ruiz et al., 2009; Ruiz et al., 2010; Santiago et al., 2010), although the consensus is that there is currently no predictive ability of genetic testing in the identification of talented sports people (Webborn et al., 2015). Bouchard et al., (2011) pooled data from three independent aerobic training programmes—HERITAGE, DREW and STRRIDE—conducted in untrained individuals, and found that those with a TGS of ≥ 19 saw VO_{2max} improvements 2.7 times greater than those with a score of ≤ 9, although this was conducted post-hoc and not used to inform programme design. As of yet, the use of a TGS or other algorithm has not been widely utilised in regard to interventions to improve exercise response. Meckel and colleagues (2014) used a TGS to retrospectively explain training response over the career of an athlete. Jones et al. (2016) used a weighted algorithm to personalise an eight-week resistance training programme; those doing genetically matched training saw significantly greater improvements in both a test of power and endurance than those doing genetically mismatched training. In addition, over 80% of athletes identified as high responders were from the matched group, whilst 82% of non-responders were from the mismatched group – suggesting that genetic testing might be useful in reducing non-response to exercise; something that will excite elite athletes, but which may also have public health connotations in the fight against obesity. Another method, utilised by Timmons et al., (2010), combined the use of RNA profiling with genetic information to create a molecular predictor of VO_{2max} response to aerobic training, although again this has yet to be satisfactory replicated (Sarzynski et al., 2016). It is still early on in this journey, and a far greater body of research is required; nevertheless, it does appear that the effective utilisation of this knowledge is drawing closer. Effective utilisation will also require manipulation of environmental factors, such as exercise intensity, duration, volume, as well as nutritional interventions; it must always be remembered that the use of genetic information can better inform how to make these manipulations, but does not serve to replace them.

The use of genetic testing within sport is still somewhat controversial (Vlahovich et al., 2017a, 2017b; Webborn et al., 2015; Williams et al., 2016), with this controversy comprised of a variety of factors. One of these regards the use of genetic testing for talent identification; currently, there is no evidence that genetic testing should be used in this way (Webborn et al., 2015). The second controversy is whether these tests have utility in terms of exercise modification. A recent consensus statement (Webborn et al., 2015) suggests that they don't, although no evidence is given in the consensus statement to support this standpoint. It's certainly true that, at present, only a small number of studies have looked at training modifications based on genetic information, but this number is expected to grow in the future, leading to the possibility that genetic information might have some use, alongside other more traditional information

sources (Grimaldi et al., 2012; Heffernan et al., 2015). Finally, there are a number of ethical issues to consider and overcome. Should there be a minimum age for genetic tests? Can the results of a genetic test be placed in the correct context for an athlete? Who owns the genetic data – the athlete or the team? If an athlete refuses a genetic test, will they be discriminated against? What happens if a genetic test unearths a potential medical issue, such as increased Alzheimer's disease risk? These questions, and others, will need to be answered before genetic testing can become widespread in sport, even though, in a recent study of elite-level athletes and coaches, the majority believed such information could be useful (Varley et al., 2018a). Finally, there needs to be assurances that the results of a genetic test will not be used for selection purposes, or any other discriminatory practices. If these ethical hurdles can be overcome, there is a potential use for genetic testing in exercise prescription and modification, alongside other more traditional aspects.

6. Conclusions & future directions

This chapter has explored some of the factors that modify the individual response to a stimulus, primarily exercise adaptation, demonstrating how the environment can affect adaptation, through aspects such as sleep and nutrition. Additionally, this chapter examined how epigenetic modifications allow communication between the environment and genetic expression. However, a constant theme throughout has been the influence of genetic factors on the response to a stimulus. Differences in genotype are responsible for a large amount of variation in exercise adaptation, but genetic factors also influence environmental aspects such as nutrition and epigenetic efficiency. Given that genetic factors are such a consistent and fundamental modulator of how someone responds to exercise, knowledge of these factors within an individual could potentially prove useful. For the first time, this knowledge is affordable and available through genetic testing, allowing athletes and coaches to have an idea of how they will respond, and to modify training to account for this. The information gained from a genetic test represents an additional piece of information to inform needs much like a vitamin D screen, heart rate variability for recovery, or a 1RM strength test. It is still early in the use of genetic testing for sports people, and a significant body of research is required to identify yet more genetic variants involved in exercise adaptation, along with other areas of interest to athletes; injury risk, recovery speed, and the ergogenic effects of nutritional aids. However, research is starting to indicate the utility of these tests. Indeed, some sports teams have been using genetic information (Dennis, 2005), but without any evidence-based practice. Given the apparent desire of high-level sports people to utilise genetic information to inform programme design, the development of evidence-based guidelines is paramount, which of course means that further research on the potential use of genetic information in training response in required, particularly from a predictive standpoint. As such, it is important for further research to focus on:

- Replication of existing, and discovery of further genetic variants that impact exercise adaptation.
- Examining the interplay between genes, environment, and epigenetic modifications on exercise adaptation.
- The development of evidence-based guidelines on the use of genetic assessments in sport, with particular reference to ethical considerations.

The ability to harness this information potentially represents a new dawn in understanding exercise adaptation, allowing athletes to better guide their quest to become faster, higher, and stronger.

CHAPTER 3 – CONTEMPORARY ISSUES REGARDING EXERCISE NON-RESPONSE & EXERCISE GENOMICS

Chapter preface:

As detailed in Chapter 2, the results of a number of studies suggest that there is the potential for considerable inter-individual variation in the response to an exercise training programme. This inter-individual variation, from a biological perspective, can be explained as the interaction between genes, environment, and epigenetic modifications (Pickering & Kiely, 2017a; Pickering and Kiely, 2018a). However, recently a number of authors have cast a skeptical eye on the data underpinning the individual response (Atkinson & Batterham, 2015; Hecksteden et al., 2015; Williamson et al., 2017; Atkinson et al., 2018; Atkinson et al., 2019). The main causes of contention are that some components of inter-individual variation are related to measurement error (Atkinson & Batterham, 2015; Hecksteden et al., 2015) or flaws in study design (Williamson et al., 2017). Such components are commonly termed "false" inter-individual variation (Atkinson & Batterham, 2015), and potentially hamper the ability to fully understand the magnitude of "true" inter-individual variation. Furthermore, there is the potential that any variability in response, whilst "true", is not clinically relevant (Williamson et al., 2018). This chapter further explores some of these issues, attempting to answer whether non-responders to exercise exist. Furthermore, as the genetic component of inter-individual variation is commonly estimated as approximately 50% (Williams et al., 2014) there is a need critically appraise some of the findings of gene-association studies to determine the validity of the findings.

PART 1 – DO NON-RESPONDERS TO EXERCISE EXIST?

1. The terminology problem: "Non-responder" vs "Did not respond"

Given the increased interest in individual variation in response to exercise, along with the potential for some individuals to exhibit no (Bouchard & Rankinen, 2001), or negative (Bouchard et al., 2012), responses, the term "non-responder" has increasingly been employed to describe those who fail to exhibit positive change in the measured variable following an intervention (Booth & Laye, 2010). The pejorative connotations implicit in such a term, however, may promote a damaging and misleading perception that exercise is perhaps not universally beneficial. This belief is potentially hugely damaging from a public health perspective, given the well-established and wide-ranging positive effects of regular exercise training on reducing obesity risk (Ross et al., 2000; Slentz et al., 2009), enhancing cardiometabolic health (Jennings et al., 1989; Grace et al., 2017), increasing function in the elderly (Li et al., 2018), improving mental health (Scully et al., 1998; Cooney et al., 2014), and reducing the risk of various disease states (Booth et al., 2012; Fiuza-Luces et al., 2013; Pareja-Galeano et al., 2015). Accordingly, it is crucial to approach such a term, and its related findings, with a critical eye. An area worthy of exploration is whether the observed non-response is modality specific. As detailed in Chapter 2, this appears to be the case. When exposed to divergent exercise stimulus, such as strength and aerobic

training, individuals appear to exhibit non-response to one, but not both, training interventions (Hautala et al., 2006; Karavirta et al., 2011). As such, exercise non-response appears to be modality-specific; and, whilst it has previously been suggested that global non-responders to exercise likely do exist (Timmons 2011), this is not currently supported by experimental data.

2. Exercise non-response: statistical insights

Given the increased interest in exercise non-response and individual variation, a number of researchers have cast a welcome skeptical eye on the underpinning data (Atkinson & Batterham, 2015; Hecksteden et al., 2015; Williamson et al., 2017; Atkinson et al., 2018). When determining whether a subject has responded to training, research designs typically require a pre- and post-intervention test, with the difference between the two test scores determining responsiveness (Hecksteden et al., 2015). However, inherent within any measurement are both technical error and random within-subject variation (Atkinson & Batterham, 2015; Hecksteden et al., 2015); such confounders are said to represent "false" individual variation (Atkinson & Batterham, 2015), potentially leading to the mis-identification of individuals as non-responders. To guard against this, a method to determine "true" individual variation has been proposed, whereby the standard deviations of the intervention group are compared to a control/comparator group, as both groups will have similar measurement error and random within-subject variations (Atkinson & Batterham, 2015; Atkinson et al., 2017). Many of the studies supporting the concept of exercise non-response, particularly with regards to aerobic training, lack such a comparator arm (Williamson et al., 2017). Accordingly, the "true" occurrence of exercise non-response may be overstated, and is currently unclear.

Furthermore, exercise non-response has no set definition; it can refer to the lack of a clinically meaningful change, the lack of a measurable change, a value above the technical error of the test, or as the lowest set percentage of participants in terms of response (Scharhag-Rosenberger et al., 2009; Vollaard et al., 2009; Bouchard et al., 2012; Hecksteden et al., 2015). This obviously makes comparisons between trials difficult, as individuals classed as a responder in one trial may be classed as a non-responder in another, thereby hampering discernment of the true rate of non-response.

There is also the potential that the type of evaluation utilised may cause differences in test performance that masquerade as individual responses. For example, maximal VO_{2max} tests are often used to determine improvements in cardiovascular fitness following training. These tests impose significant physical stress and discomfort, ensuring test performance is modulated by subject motivation (Noonan & Dean, 2000). Hence, an individual may have undergone significant physiological adaptations from a training programme, but performed poorly on the quantifying test due to motivational, non-physiological reasons. Whilst this individual would have responded positively to training, this improvement would not be reflected in test performance. This obviously has important implications for gene association studies exploring exercise non-response; for example, is a particular single nucleotide polymorphism associated with enhanced improvements in aerobic fitness, or does it merely predispose to greater exercise discomfort tolerance (Pickering & Kiely 2017b)?

Finally, the selection of tested variables appears to affect the identification of exercise non-responders. Typically, non-responders are identified within one measure, such as 1RM change or improvements in VO_{2max}. However, when data on more than one variable is collected, exercise non-response seems to disappear. For example, Scharhag-Rosenberger and colleagues (2012) had 18 previously untrained participants undergo a year-long aerobic training programme. Pre- and post-measures were collected for four variables; VO_{2max}, resting HR, exercise HR, and individual anaerobic threshold. 10 participants showed no improvement in at least one variable, but, crucially, every subject improved in at least one metric. Similarly, Churchwood-Venne et al. (2015) analysed data after participants completed a 24-week resistance training programme, collecting data on lean body mass, type I and II fibre size, and chair-rise time, along with leg press and leg extension 1RM. Again, there were non-responders for each individual measure, but no single subject exhibited non-response in all measures.

3. Should exercise non-response be a concern?

At this point, it appears that the individual variation in response to exercise is a normal and natural occurrence. This non-response has a statistical component, with the definition of non-response (Hecksteden et al., 2015), and the variables measured (Scharhag-Rosenberger et al., 2012), impacting whether an individual is labelled as a non-responder. However, at a population-wide level, by measuring only a few variables and labelling an individual as a non-responder, there is a risk of taking a reductionist approach to exercise. Exercise is commonly considered a "polypill", exerting a plethora of positive benefits (Fiuza-Luces et al., 2013; Pareja-Galeano et al., 2015), and by focusing on a small number of measures of response, it is possible to miss the bigger picture; exercise works through so many different pathways and mechanisms, that the chances of an individual exhibiting no single biological benefit is highly unlikely. Additionally, exercise clearly exerts benefits above the physiological; reducing stress and improving mental health (Scully et al., 1998; Cooney et al., 2014), as well as serving as a social aid (Hanson & Jones, 2015).

Nevertheless, some physiological measures appear to be more important than others. Timmons (2011) referred to this as an "hierarchy of health benefits", with improvements in aerobic fitness likely to have a greater bearing on health (Blair et al., 1996) and longevity (Blair et al., 1989; Kodama et al., 2009) than other measures. As such, exercise non-response in these higher-tier aspects is clearly important, as maximising the responsiveness of larger numbers of individuals to exercise could drive important improvements in population health. Additionally, when it comes to those at risk of certain diseases, chasing a response in a specific variable may be important. For example, when aiming to reduce type-II diabetes risk in a cohort of at-risk individuals, reductions in fasting glucose and/or BMI are typically prioritised (Hu et al., 2004). In this case, non-response to critical variables, and the targeting of effective exercise interventions to overcome non-response, demands greater attention.

4. "Did not respond" – potential interventions

The measurable differences in the magnitude of adaptations following an exercise training programme, if clinically relevant, raise the question "what should be done about it?" Findings from a small number of studies provide potentially important information on how best to mitigate, and potentially eliminate, exercise non-response. The simplest approach would be to undertake the training programme for longer; Churchward-Venne and colleagues (2015) reported that the longer a resistance training intervention lasted, the less prevalent non-response was, and, after 24 weeks, all participants exhibited a positive response in at least one outcome measure. Sisson et al. (2009) demonstrated that the rate of non-response decreased as exercise volume increased, from 45% at 4 kcal/kg per week (the lowest volume) to 19% at 12 kcal/kg per week (the highest training volume). Similarly, Ross and colleagues (2015) randomly assigned obese participants to different exercise protocols over a 24-week intervention; low intensity, low volume exercise (180 – 300 kcal per session at 50% VO_{2peak}); low intensity, high volume exercise (360 – 600 kcal per session at 50% VO_{2peak}); or high volume, high intensity exercise (360 – 600 kcal per session at 75% VO_{2peak}). On average, all groups increased their aerobic fitness, although there were a number of participants deemed to exhibit no response. Interestingly, there were no non-responders in the high intensity training group, demonstrating that increasing exercise intensity represents a viable method of reducing exercise non-response. Additionally, in the two low intensity training groups, the group undertaking higher total volumes had fewer non-responders (18%) compared to the group with the lower volume (39%). Furthermore, Astorino & Schubert (2014) reported that, following two weeks of low volume sprint interval training, the frequency of non-response was greater than following prolonged, high volume high-intensity training, again suggesting that exercise intensity is important. Finally, in a paper entitled "Refuting the myth of non-response to exercise training", Montero and Lundby (2017) reported that exercise non-response is dose dependent, finding that it was more likely to occur in participants exercising 1-2 times per week than in those exercising 4-5 times per week; indeed, there were no non-responders in this latter group. Furthermore, when the individuals identified as non-responders to the initial exercise intervention underwent a second intervention, identical to the first but with two additional weekly training sessions, there were no non-responders. As such, increasing exercise intensity and/or duration appear to be useful strategies for reducing, or perhaps even eliminating, exercise non-response.

A further option for enhancing training outcomes is changing training modality. Because the molecular pathways and gene networks underpinning adaptations to aerobic and resistance exercise are distinct (Baar, 2009; Joseph et al., 2006; Canto et al., 2010; Timmons 2011; Egan et al., 2013), performing exercise types that an individual can more favorably adapt to holds promise. This has been illustrated by Hautala and colleagues (2006), whereby individuals termed non-responders following aerobic training enhanced their cardiovascular fitness following resistance training. Additionally, Bonafiglia and colleagues (2016) reported that non-response to either typical endurance training or sprint interval training was largely abated when participants undertook the other exercise intervention.

Finally, there remains the possibility that, as the magnitude of exercise response is partially governed by various molecular drivers (Timmons 2011), and as these drivers are partially genetically

determined (Bouchard et al., 2011; Bouchard 2012), the use of genetic information *may* assist in the selection of more individually-optimal training prescription (Pickering & Kiely, 2017a). The potential influence of genotype on training outcomes is an emerging, currently contentious field, with both early promise (Delmonico et al., 2007; Jones 2016) and null results (Charbonneau et al., 2010). As such, the utility of using genotype to guide training interventions requires further research.

5. Summary—Do non-responders to exercise exist?

Based on available evidence, there is an individual variation in response to exercise (Bouchard & Rankinen, 2001; Hautala et al., 2005; Ahtiainen et al., 2016), with some participants experiencing larger improvements than others. This individual response is a combination of "true" and "false" variation, whereby "false" variation refers to both technical measurement error and random within-subject biological variation (Atkinson & Batterham, 2015) and "true" variation to real, between subject differences, comprised of differences in both genotype and individual history, amongst other influencing factors (Mann et al., 2014; Pickering & Kiely, 2017a; Sparks, 2017). Within published studies, there are a sub-group of individuals whom appear to exhibit either no (Ahtiainen et al., 2016) or a negative response (Bouchard et al., 2012) to exercise training programmes. The extent to which this non-response is "true" or "false" within each study remains, currently, unclear; as is whether this non-response is static (i.e., the individual will always be a non-responder to that particular exercise training programme), or merely a temporary reflection of the adaptive capacity of specific individuals at a given time (i.e., the individual did not respond to that exercise training programme, but might if the intervention was repeated). Additionally, a crucial consideration is that exercise response is often determined by measurement of one, or at most a small number, of all the potential variables that can typically change with exercise. Thus, just because an individual does not improve their VO_{2max} or 1RM with training, does not mean that they haven't derived a multitude of other benefits from exercise, many of which, such as increased social interaction seen in community exercise settings (Hanson & Jones, 2015), are non-physiological in nature.

Furthermore, there is limited evidence that increasing the number of measured variables reduces, and likely even eliminates, the prevalence of "global" exercise non-response (Scharhag-Rosenberger et al., 2012), such that it seems likely that no person exhibits absolutely no true benefit from exercise. Additionally, there is emerging evidence that the observed non-response to a single variable from a singular training intervention can be removed, either by increasing training volume, intensity, or duration (Sisson et al., 2009; Churchward-Venne et al., 2015; Ross et al., 2015; Montero & Lundby, 2017).

In summary, despite the eye-catching title of Montero and Lundby's paper on "the myth of non-response to exercise" when subjected to a standard exercise intervention, a small subgroup of individuals appear to exhibit no improvement in a given measured variable (indeed, in the initial phase of their study, there were some individuals who did exhibit no positive response to the training intervention). These individuals are commonly labelled as non-responders. However, by evaluating a greater number of variables, or by manipulating the training programme through alterations in intensity, frequency, or modality, it appears that all individuals have the potential to show improvements following exercise.

Researchers might, therefore, be better off stating that people "did not respond" to a particular intervention in a given measure, as opposed to labelling them as "non-responders", because it seems likely that a different training programme (in terms of intensity, volume, duration, or modality) would elicit a positive response. This is similar to the ideas of Booth and Laye (2010), who believed the term "non-responder" should be replaced by "low sensitivity"; in this case, these low sensitivity individuals merely require increased volumes and/or intensity to drive a favourable response. Undoubtedly, this is a positive finding, given the wide ranging benefits of exercise on health and wellbeing; however, further research is required to identify the optimal way to align individuals to the training type most likely to elicit the greatest adaptations, especially given the limited time many people perceive they have available to exercise, along with concerns about the applicability of increased exercise intensities for all exercisers (Biddle & Batterham, 2015). Furthermore, future research should focus on predicting who will exhibit a lower response to exercise, so that they can be given an alternative, more efficacious training intervention. Such research has the potential to have a huge impact on the heath of populations, increasing the health and fitness of time poor individuals in a more effective manner.

PART 2 - EXERCISE GENETICS: SEEKING CLARITY FROM NOISE

1. Introduction

Having discussed whether non-responders to exercise exist, a second issue emerging within contemporary exercise genetics research is that of signal and noise; are the associations found within studies true, or spurious? Unravelling the genetic underpinnings of exercise performance is currently a hot topic in sports science and medicine, which, although controversial (Webborn et al., 2015), has extensive potential implications. One such potential application is the use of genetic information to enhance exercise prescription, thereby positively influencing athletic performance and public health domains. Recent research suggests that this is both feasible and potentially beneficial (Timmons et al., 2010; Jones et al., 2016). However, the effective use of genetic information often requires a clear understanding of the mechanism by which each reported single nucleotide polymorphism (SNP) mediates physical performance. One such problem area is that of genetic association studies, where a SNP or group of SNPs are associated with a trait, such as VO_{2max} improvements. Whilst an association is suggestive, the question remains as to whether this association is potentially causative, or spurious. If spurious, then the subsequent conclusions and exercise prescription are potentially misleading. The following sections highlight some complexities evident within this realm, illustrating the need for further research.

2. Association or causation?

Within the HERITAGE Family Study, variation in *CREB1* (rs2253206) was predictive of the heart-rate (HR) response to exercise (Rankinen et al., 2010). Specifically, the A allele was associated with a smaller reduction in HR during a sub-maximal exercise test following training, with the proposed mechanism relating to long-term cardiac memory. However, research in a separate cohort associated the

A allele with a greater exercise-induced temperature increase, contributing to a less pleasant subjective experience of exercise, potentially reducing motivation to train or carry out an aerobic test (Karoly et al.., 2012). Accordingly, it is unclear whether HR-responsiveness was modified via biologically-mediated adaptations, or an increased perception of effort.

Similarly, a SNP within *COL5A1* (rs12722) has been linked to exercise-associated muscle cramps (EAMC), with the CC genotype associated with protection from EAMC during an ultra-marathon (O'Connell et al., 2013). However, CC genotypes also recorded significantly slower ultra-marathon times compared to TT genotypes (O'Connell et al., 2013). Does this genetic variation directly protect against EAMC, or, does it result in slower race times? This latter point is important; as EAMC is associated with increased neuromuscular fatigue (Bergeron 2008), is this what acts in a protective manner? Again, the biological impact of this SNP on EAMC isn't clear, requiring more evidence before advice can be given.

A final example is that of *TTN*, the gene which encodes for titin, a protein found within striated muscle cells. A SNP within this gene was found to affect maximal oxygen uptake response to exercise in HERITAGE (Rankinen et al., 2003), the proposed mechanism being that this variation affects the elasticity of cardiac muscle, impacting stroke volume (SV). This increase in SV enhances oxygen delivery to the muscles, improving aerobic fitness. However, the same SNP has been found to modify muscle fascicle length and marathon time in marathon runners (Stebbings et al., 2017). Here, the T allele is associated with shorter muscle fascicles, which is hypothesised to increase the efficiency of the running action. In this case, the SNP does not directly affect aerobic fitness, but running economy. Either or both proposed mechanisms may be correct, but greater certainty of the biological mechanism is required before training intervention advice can be given.

3. Are these relationships consistent?

In addition to resolving the biological mechanisms underpinning the impact of genetic variation on exercise, it is crucial to also consider whether these genetic associations are consistent over time and across different cohorts. As demonstrated in part one of this chapter, much is made of non-responders to exercise, and yet it's not clear whether this non-response is consistent, or whether it is a one-time response to an intervention. In addition, it's unclear whether SNPs associated with exercise response in sedentary individuals have similar effects in trained individuals. A SNP in *ACSL1*, rs6552828, had the strongest association with training-induced VO_{2max} improvements in HERITAGE (Bouchard et al., 2011), a sedentary cohort. However, in an elite athlete cohort, there was no association between this SNP and elite endurance status (a proxy of high VO_{2max}) in Caucasians (Yvert et al., 2012) or Israelis (Ben-Zaken et al., 2018). No further *ACSL1* replications exist. Does variation in *ACSL1* impact exercise adaptation in all humans, or only the subset of humans who took part in HERITAGE? If HERITAGE were to be repeated with the same participants, would the *ACSL* and aerobic fitness association remain constant? Does this variation affect trained and untrained individuals to the same extent? Answers to these questions are needed before these SNPs should be used to modify the training process.

4. Effective utilisation

Despite these issues, there are a number of SNPs in which the biological mechanisms are well understood. A common SNP in *ACTN3*, the gene that encodes for a-actinin-3, a protein found exclusively in fast-twitch muscle fibers, results in a premature stop codon. Individuals homozygous for this polymorphism are unable to produce the protein, and as a result tend to have fewer fast-twitch fibers (Vincent et al., 2007). This in turn affects the response to strength training (Delmonico et al., 2007). The utilisation of this information holds promise; a recent paper used this SNP in conjunction with fourteen others to enhance resistance training response (Jones et al., 2016), and evidence-based guidelines have been proposed (Kikuchi & Kakazato, 2015). These initial findings underscore both the effectiveness and utility of genetic information in informing training methodologies *when* the biological mechanism is well understood.

5. Summary

Research into the genetics of exercise adaptation is both exciting and promising. However, whilst genetic associations are interesting, their underpinning biological mechanisms must be understood before the information can be utilised in training programme design. As exemplified in the examples of *CREB1, TTN* and *COL5A1*, it may be that each SNP has multiple mechanisms, or the gene-trait association may be spurious, and hence misleading. Elucidation of this is crucial, and when it comes to interpreting the results of gene-association studies, practitioners should be mindful that association is not evidence of causation, with a host of confounders potentially skewing the findings. Perspectives on the promise of exercise genetics vary widely, with polarised extremes of staunch advocates and deniers. For the majority, the complex relationship between genotype and phenotype promotes a healthy skepticism; nevertheless, a total rejection of the potential utility of gene panels to categorise adaptive sub-types, given promising preliminary findings (Timmons et al., 2010; Delmonico et al., 2007; Kikuchi & Nakazato, 2015; Jones et al., 2016), is premature. Beyond a formulaic statement of the obvious—that correlation is not causation—it seems wise to proceed cautiously, skeptically, but with an open mind as more evidence unfolds.

CHAPTER 4 – METHODOLOGY

Chapter preface:

As discussed within Chapters 2 and 3, there is considerable inter-individual variation in response to a stimulus. This was explored in detail with regards to the individual response to exercise, concluding that, in terms of the "true" biological variation between individuals who are subjected to an identical training programme, these differences can be thought of as either genetic, environmental, or epigenetic in nature. Estimates of the genetic component of individual response vary depending on the trait, but, on average, around 50% of the inter-individual variation in exercise response is thought to be driven by genetic variation. As the genetic component is both a large and fundamental modifier of the training response, there is the potential that knowledge of an individual's genetic make-up may aid in training programme design. The aim of this thesis is to explore this from the perspective of elite sport, and this chapter will explain the methodologies utilised to achieve this aim.

1. A brief history of sports genetics research – from twins, to candidate gene association studies, to GWAS.

Research into the realm of the genetic underpinnings of exercise initially focused on a combination of family and twin studies. Twins are a useful proposition for genetics research, because monozygotic (MZ) twins have (essentially) identical genotypes, whilst dizygotic (DZ) twins share only half of their DNA in common. As a result, it is possible to collect information on a trait, and compare the between twin-pair variation for both MZ and DZ twins. By dividing the difference of the variance between DZ and MZ twins by the variance of DZ twins, it is possible to determine the heritability estimate (h^2) of that trait (Wang et al., 2013). Such an approach was utilised in the early days of sports genetics research with an aim to determine the heritability of VO_{2max} (Williamson et al., 2017). Subsequent research utilising twin models determined the h^2 of many traits, including the heritability of elite athlete status, which was estimated at 66% (De Moor et al., 2007). However, the often unrealistically high h^2 value attributed to traits, such as 83% for muscle strength (Beunen & Thomis, 2004), lead to criticisms of this approach.

Over the following years, improved gene sequencing ability, coupled with a lowering in cost of the process, lead to an increase in gene-association and candidate gene analysis studies. Here, researchers have a prior hypothesis that a specific genetic variant impacts a specific trait, and then test that hypothesis. Typically, this occurs through a case versus control analysis, whereby individuals with the phenotypic trait of interest are compared to individuals without that trait. For example, in order to determine whether gene X is associated with type-II diabetes, researchers would collect genetic information from a group of type-II diabetics, and compare the relative genotype frequencies for gene X in a normal, non-diabetic population. Such an approach is certainly valid, especially when there is a plausible underlying mechanism, although it does rely on the prior identification of potential candidate genes (Wang et al., 2013; Bouchard, 2015).

Due to the requirement for a prior understanding of which SNPs may be associated with a specific trait within gene-association studies, when it comes to attempting to identify novel genes associated with that trait, there has been a move towards Genome-Wide Association Studies (GWAS). Here, large numbers of SNPs (100,000+) are compared between cases and controls, allowing for an increased chance of the discovery of novel SNPs, given that such an approach is hypothesis free. However, as the many genetic variants underpinning a phenotype likely have a small effect size, and, due to the number of comparisons that are tested resulting in the genome-wide significance level of a p-value of $<5 \times 10^{-8}$, GWAS require very large cohorts. As such, the utility of GWAS when determining trait associations for relatively rare phenotypes, such as elite athlete status, is limited, given the low number of individuals with the required traits.

2. Just how much utility does current sports genetics research bring?

Based on evidence reviewed in Chapters 2 and 3, it's clear that genetic variation has a role to play in a number of traits pertinent to elite sport. However, at present, it is not clear just how useful this information is to athletes and practitioners in its current format. Recently, Varley and colleagues (2018a) reported the results of a survey exploring the prevalence of genetic testing within elite sport in the UK, and the opinions of athletes and their support staff towards the use of such tests, which, given the on-going debate around the use of genetic tests in sport (Webborn et al., 2015; Vlahovich et al., 2017a, 2017b) was both timely and relevant. The results themselves were interesting, with almost all (97%) of the support staff and 79% of the athletes expressing the belief that genetics played some role in the development of elite athletes. Interestingly, whilst the majority of support staff (~72%) felt that genetic testing would be useful, and most athletes (~79%) would be willing to take such a test, the prevalence of genetic testing at this level was reportedly low (≤17%). As such, there appears to be a relative mis-match between the perceived utility of genetic testing in elite sport, and the actual uptake. The authors did not explore the underpinnings of this mis-match, which sensibly requires further research.

One obvious potential explanation, however, is the perceived lack of evidence supporting the utility of genetic testing within elite sport contexts. At present, the majority of studies exploring the genetic influence on exercise tend to focus on *explaining* the variation, as opposed to attempting to *utilise* this information. For example, whilst it is well-supported that a polymorphism in *ACTN3* has a well-replicated effect on speed-power athlete status (Yang et al., 2003; Druzhevskaya et al., 2008; Papadimitriou et al., 2016) as well as modifying the response to a resistance training programme (Delmonico et al., 2007), so far only one study (Jones et al., 2016) has used information on participants' *ACTN3* genotype, as part of a panel of 15 SNPs, to guide training programme design. Similarly, whilst researchers have identified a number of SNPs associated with injury risk (Mokone et al., 2006; Posthumus et al., 2009a, 2009b, 2009c; Ficek et al., 2013), as of yet no genotype-based interventions have been made in an attempt to reduce the occurrence of injuries.

Furthermore, at present the majority of the explanatory studies tend to focus on single SNPs. As

each SNP may only have a small effect size, the utility of such information is perhaps limited. As a result, a potential solution is to utilise Total Genotype Scores (TGS), which are explored in greater detail later in this chapter. Such an approach combines the results for a number of genetic variations into a single output, which should explain a greater amount of the variance than single SNP information does.

3. Bridging the gap?

If a perceived lack of utility to genetic information in elite sport is preventing increased uptake of such information, then an important next step is for researchers to bridge this gap by supplementing more observational research, such as gene-association studies, with hypothesis-driven intervention studies. Such studies are needed to test the practical utility of genetic information, not just in elite sport, but across the spectrum of physical abilities and activity levels. There is scant research exploring the use of genetically guided interventions within sport, with only one study, to the present authors knowledge, taking such an approach (Jones et al., 2016). Although this research was well received (Monnerat-Cahli et al., 2017), the study utilised a commercially available test, and clearly it is appropriate that scientists and practitioners view research conducted by such commercial companies with a healthy scepticism. Nevertheless, it is important to be both pragmatic and open minded; if athletes and sports teams do engage in genetic testing, which most seem amenable to (Varley et al., 2018a), then potentially the most readily available avenue to pursue is via commercial products. This is particularly true for assessments screening for multiple SNPs associated with specific phenotypes, as is the case with TGS and genetic-based algorithms. Given that both exercise response and injury risk—the most popular proposed avenues for the use of genetic information within sport—are polygenic traits, such approaches appear the most promising route forward. As such, by virtue of providing a testable product utilising TGS/genetic-based algorithms, direct-to-consumer (DTC) companies perhaps should form part of this evidence gathering process, either by partnering with more traditional research outlets, or by subjecting their own internal studies to the peer-review process. Such research partnerships have previously been utilised by 23andme, a DTC genetic testing company based in the U.S., which has explored the genetic basis of numerous pathologies (Chang et al., 2017; Ferreira et al., 2017).

Interventional research, although necessary, can often be problematic, particularly in elite sport contexts. Genetics-based studies often require large numbers of participants to uncover genotype-phenotype associations, and typically—and indeed by definition—there is a limited availability of elite athletes within any population. Similarly, sports practitioners and athletes arguably do not care about gene association studies conducted in non-elite cohorts, in which the environmental conditions, such as training history and lifestyle, are inevitably different than in elite athletes (Buchheit, 2017). To overcome such limitations within genetic intervention studies, researchers should perhaps place elite athletes into sub-groups based on their TGS, and treat each group as discrete from one another. Such an approach, whilst not perfect for genetics research, at least makes such intervention studies more practically viable with the subject numbers most commonly found in professional sports teams – and, indeed, is an approach common in sports science research.

4. The DNAFit test

One potential issue with the use of a TGS is that of practicality; how does the user in the field—the athlete, coach, or practitioner—develop a TGS for their use, and then collate that information? Doing so would be very difficult; many coaches and sports practitioners are not experts in genetics, and nor should they be. However, such a lack of expertise makes it difficult for them to develop an optimal TGS for their needs. Additionally, the collection of genetic information outside of universities, without relying on a commercial company to do so, is practically difficult. Fortunately, some commercial genetic testing companies utilise TGS in the reporting of their data, and, as discussed in section 3, the use of such commercial companies to undertake both genotyping and analysis represents a potential avenue for exploration. Commercial companies, by virtue of having the required technology and refined reporting techniques, have a testable product that may hold utility to those in the field; it is important, therefore, to test these products to determine if they have utility to athletes and their support staff. This is one of the undertakings of the present thesis, and the company providing genetic testing will be DNAFit Life Sciences (London, UK).

DNAFit provide genetic testing services through different laboratories depending on the depth of data required. Both methods require the same method of DNA sample collection, which is via a sterile buccal swab. For smaller SNP (n=~50) analysis, the samples are sent to IDna Genetics Laboratory (Norwich, UK), where DNA is extracted and purified using the Isohelix Buccalyse DNA extraction kit BEK-50 (Kent, UK), and amplified through PCR on an ABI7900 real-time thermocycler (Applied Biosystem, Waltham, USA). For larger SNP (n=~700,000) analysis, the samples are sent to AKESOgen, Inc (Peachtree Corners, GA, USA), where DNA is extracted from the saliva samples using Qiagen chemistry on an automated Kingfisher FLEX instrument (Thermo Fisher Scientific, Waltham, MA, US), following the manufacturer's recommended protocols and standard operating procedures. PicoGreen and Nanodrop measurements are taken to measure the quality and quantity of the DNA. Input to the custom testing array occurs at 200ng in 20μL. Amplification, fragmentation, and resuspension are performed using Biomek FXP following Affymetrix's high throughput protocol for Axiom 2.0. Hybridisation is performed for 24 hours at 48°C in a Binder oven, and staining and scanning of the arrays are performed using GeneTitan instrumentation (Thermo Fisher Scientific, Waltham, MA, US), all following the same Affymetrix high throughput Axiom 2.0 protocol. Data analysis can then be performed using a raw CEL file data input into the Affymetrix Axiom Analysis Suite (Affymetrix, Santa Clara, CA, US).

The reliability of each laboratory testing method is internally assessed via DNAFit. The company holds a number of anonymised genetic samples, which it sends to different laboratories. The results of each laboratories analysis are then compared to previous analysis, either from that laboratory or other laboratories, to check for anomalies. Both the IDna and AKESOgen methods have been well-tested using this methodology, with a number of different samples across different time points. The laboratories provide reliable results which are concordant with their previous analysis, and the analysis from other providers. As such, the genetic analysis provided by both methods is believed to be reliable and accurate.

Once the raw data is received from the respective laboratories by DNAFit, it is then populated into set reports through automated software. At the time of writing, DNAFit provide two major report categories; one for fitness, and one for diet. In selecting the SNPs to report on in each report, DNAFit require a minimum evidence threshold to be met. Each SNP should have a minimum of three peer-reviewed studies, conducted on humans, showing a consensus as to the effect of that SNP. Finally, DNAFit specifically will not report on genetic variants that are considered to be related to a serious medical condition, such as cancer (e.g. *BRCA* genes and breast cancer [Miki et al., 1994]) or Alzheimer's Disease (e.g. *APOE4* [Sanan et al., 1994]).

All of the fitness related traits are reported by utilising a Total Genotype Score (TGS). Of interest in this thesis will be the Aerobic Trainability and Recovery Speed TGS sections of the report, which are experimentally explored in Chapters 10 and 11. The use of TGS as opposed to the reporting of single SNPs has the potential to increase both the utility and accuracy of the information provided. Such an approach—termed a "polygenic profile"—was first utilised by Williams and Folland (2008). As individual polymorphisms only have a small impact on particular phenotypes, the authors suggested combining multiple polymorphisms into a total score in order to better understand how such polymorphisms may contribute to performance. Here, typically the most favourable performance genotype for each SNP was given a score of 2, with the least favourable genotype a score of 0. Heterozygotes (i.e. those with a favourable and unfavourable allele) were given a score of 1. The scores were then added, divided by the total possible score, and multiplied by 100 to get a percentage. As such, an individual with a TGS of 100 would have had a "perfect" polygenic score, whilst a score of 0 would represent the worst possible score. Other studies have taken a similar approach, both for exploring SNPs associated with elite athlete status (Ruiz et al., 2009; Santiago et al., 2010), the magnitude of post-exercise muscle damage (Del Coso et al., 2018a), and, in a wider context, for disease prediction (Meigs et al., 2008; Dudbridge, 2013).

For the Aerobic Trainability and Recovery Speed TGS sections of the commercially available DNAFit report, previous analysis (https://blog.dnafit.com/am-i-normal-aerobic-trainability and unpublished data by Pickering) has provided some information around the relative frequency of different outcomes. These relative frequencies were calculated from 17,000 customer samples collected from 2013-2016, which were de-identified and analysed by the author as part of his employment. This information is referenced in Chapters 10 and 11 when placing the study results in context.

5. Why use Total Genotype Scores?

The use of TGS for the reporting of information to athletes, coaches and support staff holds some potential advantages. Most traits are polygenic in nature, meaning that a large number of genetic variants likely contribute to the observed phenotype. For example, when it comes to adaptations observed following resistance training, variation in genes across a number of adaptive pathways could modify the response (Timmons, 2011). This variation could be within the mTORC1 pathway, a molecular driver of

hypertrophy (Drummond et al., 2009; Timmons 2011), or within the number of available satellite cells (Petrella et al., 2008). Variation could occur on a structural level, such as in the type and relative proportions of muscle fibre, which may respond differently to imposed training loads (Fry, 2012). It could also be down to variations in anabolic hormone concentrations, such as testosterone (Basualto-Alarcon et al., 2013), which again will modify the potential hypertrophic response. By focusing on just a single pathway, or, even more commonly, just a single gene, there is a likelihood of misunderstanding the complexities of adaptation. Furthermore, because exercise adaptation is a polygenic trait, an individual will almost certainly possess more favourable versions of some SNPs, and less favourable versions of others. By reporting individual SNPs, this can lead to confusion to the end-user. For example, some DTC genetic companies do report these SNPs individually; by uploading genetic data to one such company, an athlete may get the following advice; for SNP1 "would likely respond better to power-based training"; for SNP2 "would likely respond better to endurance-based training"; for SNP3 "would likely respond better to endurance-based training", etc. As such, the athlete will be confused – which training type would they respond better to? A TGS can overcome this by combining the results into a single, hopefully usable, output for the person who needs to utilise the information.

An example of the potential utility of a TGS, as opposed to single SNP reporting, was provided by Jones and colleagues (2016). Here, the authors gave 67 participants a DNA test, and determined their results for 15 SNPs thought to impact the response to power- or endurance-based training. A TGS was then calculated that balanced the scores for alleles thought to confer greater improvements from power- and endurance-based training, and reported as a balanced percentage. The participants were determined to be those who would respond more favorably to power (balanced percentage score of >50%) or endurance (balanced percentage score of <50%) based training. They were then randomised to receive either a genetically matched (i.e. those predicted to respond favourably to power-based training received power-based training) or mismatched (i.e. those predicted to respond favourably to power-based training received endurance-based training) eight-week resistance training programme, with a pre- and post-intervention testing session comprised of countermovement jump (CMJ) and Aero3 (maximum 3-minute cycle) tests. The results suggested that those undertaking genetically matched training saw much greater improvements than those following mismatched training.

6. Methodology for thesis

As discussed previously, one of the main challenges currently facing sports genetics research is the potential usability of the information gained from such tests. At present, the majority of the research appears to focus primarily on single gene-associations, although there have been recent papers utilising TGS to determine the magnitude of post-exercise muscle damage (Del Coso et al., 2017a, 2018a). Furthermore, there is a movement towards the utilisation of GWAS studies, generally seen as the gold standard in genetics research (Wang et al., 2013). However, whilst such studies help explain the differences between people, what they don't do is provide usable information to practitioners and information in real-world terms. This is the main area for exploration in the present thesis; an attempt to

move away from explaining the differences between athletes, and towards being able to use that information as a method to enhance performance.

In order to explore the use of genetic information in elite sport, my thesis is split into two main sections. Section 3, entitled “Joining the dots”, is a theoretical exploration of the potential use of genetic information within sport. This will be across multiple realms, including the use of caffeine as an ergogenic aid and the reduction of hamstring injuries, but all have the overarching goal of enhancing the performance of a given athlete. These chapters focus heavily on bringing together information gleaned from previous research, and attempting to make connections that have not previously been made. As such, the main methodological considerations for this section are the requirements for a thorough review of the literature. This will take place through structured literature review via established databases such as PubMed. Having reviewed and understood the underpinning evidence, the next section attempts to “join the dots” between genetics and performance, making suggestions for how this information could be used to enhance performance, and which directions future research will need to travel in order to enhance knowledge in this area.

Section 4, entitled “Practical use of genetic information in sport”, is the section containing novel experimental data. Chapter 9 focuses on reporting the results of an internet-based questionnaire exploring the attitudes towards, and prevalence of, genetic testing in athletes, coaches, and support staff. The online questionnaire was comprised of a maximum of 44 potential questions, with participants directed towards specific questions based on their answers. The full questionnaire can be found in the appendix. The link to the online questionnaire was shared via social media accounts (both on Twitter and Facebook) in order to ensure maximal coverage. The questionnaire was anonymous in order to reduce any perceptions that the information could be used for marketing by DNAFit, which would represent a significant conflict of interest. Upon completion of the questionnaire, which ran for three months, the answers were collated into a database for qualitative analysis. Further in-depth methodological details are found in the chapter itself.

Chapter 10 reports on the results of a study aimed at determining whether the use of a five SNP TGS could help identify those individuals likely to exhibit the greatest and smallest improvements following a standardised aerobic training programme. Here, 42 male soccer players aged 16-19 years volunteered for and gave informed consent to take part in the study, and provide genetic information. The players were from a convenience sample of a college soccer academy that had an agreement in place with DNAFit Life Sciences to conduct genetic testing. The sample size of 42 was chosen as it best represents the size of a typical soccer squad (first and reserve team), providing the real-world utility that the present thesis aimed to explore. The participants were well trained, with an average of 11 years’ playing experience. Both prior to, and immediately after, an eight-week aerobic training block, the players underwent an aerobic fitness assessment. The test utilised was the Yo-Yo Intermittent Recovery Test, Level 1 (Yo-Yo IR1), which has previously been shown to be both a reliable and valid measure of aerobic fitness (Krustrup et al., 2003). The aerobic training intervention was an eight-week block of two weekly training sessions primarily comprised of small sided games, which represent a sport specific method of enhancing aerobic fitness (Impellizzeri et al., 2006). Each session was comprised of four sets of four-

minute exercise blocks, interspersed with three-minute recovery periods. Genetic information was determined via the IDna method detailed above, and genetic information was determined for five SNPs; *PPARGC1A* (rs8192678), *VEGF* (rs2010963), *ADRB2* (rs1042713 and rs1042714), and *CRP* (rs1205). These SNPs were chosen as they fulfilled the DNAFit inclusion criteria, and the scores on each were combined in a weighted algorithm format to create a TGS. Such a process has been used in previous studies (Ruiz et al., 2009; Meckel et al., 2014; Jones et al., 2016). Based on their TGS, participants were then stratified into three groups; "low", "medium", and "high", with the higher score indicating the possession of a greater number of SNPs considered favorable for adaptation. In terms of statistical analysis, a mixed methods ANOVA was run to determine group changes in Yo-Yo score, with individual t-tests used to determine differences between groups. Effect sizes (Cohen's d) were also calculated for both within- and between-group differences. Full methodological details are contained within Chapter 10.

Chapter 11 focuses on a study exploring the impact of genetic information on determining the recovery speed of individual athletes. Here, 18 male soccer players aged 16-19 years of age underwent a repeated sprint session of two sets of seven 25m sprints, with 30 seconds recovery between sprint reps, and 5 minutes recovery between sets. Immediately prior to, immediately upon completion, and at 24 and 48 hours following the repeated sprint session, the participants underwent countermovement jump (CMJ) testing as a measure of neuromuscular fatigue. The CMJ has previously been shown to be a reliable and valid measure of such fatigue (Cormack et al., 2008a, 2008b, 2008c; McLean et al., 2010; Gathercole et al., 2015), and is widely used within sporting settings (Taylor et al., 2012). At each time point, the participants underwent three CMJ trials, separated by two minutes recovery. The score of the trials were averaged to give a mean score. As per Chapter 10, genetic testing was conducted utilising the IDna method outlined earlier. Genetic information was determined for seven genetic variants thought to influence post-exercise recovery speed; *CRP* (rs1205), *GSTM1* & *GSTT1* INDEL, *IL-6* -G174C (rs1800795), *IL-6R (*rs2228145), *SOD2* (rs4880), *TNF* G-308A (rs1800629). Using a proprietary algorithm from DNAFit Life Sciences, the participants genetic results were applied to a weighted TGS, and, based on the results of this TGS, participants were assigned to "slow", "medium", or "fast" recovery speeds. Such a method has been previously utilised in published research on exercise-induced muscle damage (Del Coso et al., 2017a; 2018a) and physical performance (Ruiz et al., 2009; Meckel et al., 2014; Jones et al., 2016). Given the small sample size of this cohort, and given that typical null hypothesis significance testing is sensitive to such small sample sizes (Buchheit 2016), only effect sizes (Cohen's d) were calculated for difference between the groups at all three post-training time points. Full methodological details of this study are contained in Chapter 11.

Chapter 12 reports on the extended genotype data of a number of elite athletes, including an Olympic Champion, an Olympic Medallist, and a World Championship medallist. Here, participants were contacted to determine their interest in taking part in such a study, giving informed consent and providing a DNA sample. In this case, the DNA was analysed via the Akesogen method detailed above, allowing for a richer data set of up to 700,000 SNPs to be determined. Such a richer data set allows for deeper analysis than in currently commercially available from DNAFit Life Sciences via the IDna method, and was required in order to explore a greater number of SNPs that may contribute to performance. For each athlete, their genetic results were then compared to a number of established markers for elite athlete

status, primarily derived from two recent review articles (Ahmetov & Fedotovskaya 2015; Ahmetov & Egorova, 2016). These results were then compared to a control population, in order to discover whether the athletes represented genetic outliers in terms of their TGS, which is what would be required to use genetic information to identify future talented performers. Full methodological details of this study are contained in Chapter 12.

Following these two sections, in which the use of genetic information in sport will be both discussed and experimentally explored, there will be a final section. This section contains a discussion around the wider, public-health related aspects of exercise genomics, a chapter examining a personalised training framework, and a final chapter, which serves as a discussion of the findings of the thesis, culminating in gaining an answer to the question of "is there utility to genetic information in elite sport?"

7. Summary

As discussed in this section, over the years the exploration of genetics within sports and exercise has shifted from a gross, blunt tool in the form of twin studies, to the precise use of single SNP association studies that test a specific hypothesis. More recently, as technology has evolved, there has been a shift towards Genome-Wide Association Studies (GWAS), which are used in the discovery of novel genotype-phenotype associations in a hypothesis-free approach. However, so far, the evidence base tends to be focused on the identification of genetic variants that help to explain the variation between people. Whilst such information is useful in enhancing the knowledge base, it is arguably less useful for people involved in the field; athletes, coaches, and support staff. Research suggests these individuals are amenable to utilising genetic information, but many have yet to do so (Varley et al., 2018a). The reasons for this are currently unknown, which is why they are explored in Chapter 9. However, one potential reason for the lack of utilisation of genetic information is that, in its present format, there is little evidence of its utility. Chapters 5-8 explore potential avenues for the use of genetic information to enhance the sports performance and preparation processes. Additionally, in order for genetic testing to become more useful to the people who will be using it, there is a need for an increased emphasis on multi-gene models, such as Total Genotype Scores, which combine the results of many genes into a single output. At present, the only way to gain such information outside of a university partnership is through commercial, Direct-to-Consumer genetic testing companies. As such, a commercially available test, that has a number of TGS already developed, will be explored in Chapters 10 & 11. Finally, Chapter 12 applies a TGS to a small group of elite athletes, and compares their scores against a normal population, to determine whether their results could be used as a talent identification tool. As such, the totality of this work should represent the first steps towards a more effective utilisation of genetic information within sport.

SECTION 3 – JOINING THE DOTS: A THEORETICAL APPROACH TO THE UTILISATION OF GENETIC INFORMATION IN SPORT

The content of this section draws on six previously published peer-reviewed papers, along with additional work. The published papers are:

Pickering C, Kiely J. Are the current guidelines on caffeine use in sport optimal for everyone? Inter-individual variation in caffeine ergogenicity, and a move towards personalised sports nutrition. Sports Med. 2018;48(1):7-16.

Pickering C, Kiely J. ACTN3: More than just a gene for speed. Front Physiol. 2017;8:1080.

Pickering C, Kiely J. Can the ability to adapt to exercise be considered a talent—and if so, can we test for it? Sports Med Open. 2017;3(1):43.

Pickering C. Caffeine, CYP1A2 genotype, and sports performance: is timing important? Ir J Med Sci. 2018; doi: 10.1007/s11845-018-1811-4

Pickering C, Kiely J. Hamstring injury prevention: A role for genetic information? Med Hypotheses. 2018;119:58-62.

Pickering C, Kiely J, Grgic J, Lucia A, Del Coso J. Can genetic testing identify talent for sport? Genes. 2019b;10(12):972.

CHAPTER 5 - ARE THE CURRENT GUIDELINES ON CAFFEINE USE IN SPORT OPTIMAL FOR EVERYONE?
INTER-INDIVIDUAL VARIATION IN CAFFEINE ERGOGENICITY, AND A MOVE TOWARDS PERSONALISED SPORTS NUTRITION

Chapter preface:

Caffeine is a widely used ergogenic aid at all levels of sport. However, as discussed in the introduction to this thesis, there is considerable variation in how individuals respond to caffeine, both in terms of performance enhancement, and regarding issues such as sleep disturbances and anxiety. This chapter explores the evidence demonstrating the inter-individual variation in caffeine ergogenicity, as well as the underlying drivers of this variation. Building on this, it then provides a rationale for the future potential use of genetic information in the development of personalised caffeine guidelines within sport. This chapter draws on a paper published in *Sports Medicine* (Pickering & Kiely, 2018a), which was the first to review the observed inter-individual variation seen following caffeine ingestion, as well as the underlying causes of this variation.

1. Introduction

1,3,7-trimethylxanthine (caffeine) is one of the most widely used performance enhancing drugs. Between 1984 and 2004, caffeine was banned for in-competition use, although only at very high doses ($12\mu g.ml^{-1}$). Nevertheless, this did not deter athletes, with research demonstrating that 74% of samples tested via the anti-doping process contained measurable levels of caffeine (Van Thuyne et al., 2006). Since the removal of the ban, caffeine use has remained consistent, with measurable levels found in 74% of samples between 2004 and 2008 (Del Coso et al., 2011), illustrating that the use of caffeine is widespread in athletic populations.

The performance enhancing effects of caffeine have been known for over 100 years (Rivers & Webber, 1907), and it is now a well-established ergogenic aid, with performance-enhancing effects confirmed at meta-analysis level (Grgic et al., 2019). These ergogenic effects are present across a variety of exercise types, including aerobic and muscular endurance, anaerobic power, speed, and jumping performance (Burke, 2008; Glaister et al., 2008; Astorino & Roberson, 2010; Duncan et al., 2013; Da Silva et al., 2015; Polito et al., 2016; Grgic et al., 2018; Grgic et al., 2019; Grgic & Pickering, 2019), whereas its impact on maximum strength is less clear (Beck et al., 2006; Goldstein et al., 2010a; Eckerson et al., 2013).

Caffeine exerts its ergogenic effect via several different proposed mechanisms. Within the central nervous system (CNS), caffeine acts as a competitive adenosine receptor antagonist (Urry & Landolt, 2014), thereby reducing adenosine's downregulation of arousal and nervous activity (Ribeiro & Sebastiao, 2010). Additionally, the binding of caffeine to adenosine receptors increases neurotransmitter release and muscle firing rates (Kalmar 2005). Caffeine also stimulates adrenaline secretion (Graham 2001), alters substrate utilisation (Cruz et al., 2015), increases cellular ion release (Sokmen et al., 2008) and decreases pain perception (Laurent et al., 2000; Gonglach et al., 2016), all of which can improve exercise performance.

Elevated caffeine concentrations appear in the bloodstream as quickly as 15 minutes post-ingestion, peaking after about 60 minutes, with a 3-to-4-hour half-life (Graham 2001). Caffeine is primarily metabolised in the liver, almost exclusively by Cytochrome P450 enzymes, into paraxanthine, theophylline, and theobromine (Tang-Liu et al., 1999); these in turn may mediate some of caffeine's performance enhancing effects (Graham 2001). There remains the possibility that caffeine metabolism also occurs with the Central Nervous System (CNS), although this has been primarily studied in animal models (Fredholm et al., 1999). There is also evidence of Cytochrome P450 expression and activity within the CNS, raising the possibility that localised CNS caffeine metabolism is partially mediated by these enzymes (Dutheil et al., 2010). However, overall the pharmacokinetics of caffeine metabolism within the human CNS are poorly understood at present.

Typically, generalised guidelines recommend ingestion of 3-9 mg/kg of caffeine approximately 60-minutes prior to exercise, and suggest there are no additional benefits associated with higher doses (Ganio et al., 2009; Goldstein et al., 2010b; Brooks et al., 2016). However, recent research has illustrated that ergogenic effects of caffeine can occur with a wide variety of caffeine doses and timings. For example, a recent review (Spriet 2014) focused on the effects of low doses of caffeine (< 3 mg/kg) on performance enhancement, finding that lower intakes of caffeine do tend to exert ergogenic effects. However, it isn't clear whether these effects are equivalent to those seen with doses of 3 mg/kg or above. In relation to optimal timings of intake, Cox and colleagues (2002) illustrated that 6 mg/kg of caffeine consumed 60-minutes prior to exercise was no more effective than six doses of 1 mg/kg of caffeine spread throughout the exercise bout. Accordingly, at least in some longer duration athletic events, caffeine ingestion during the event may be advisable. The prevalent use of caffeine within sport, and the assumed universal applicability of these generalised caffeine guidelines, seem to suggest there is a standard, predictable response to caffeine across individuals. This chapter discusses why this is not the case, and illustrate that, in fact, there is considerable inter-individual variation in the ergogenic effects of caffeine ingestion. This chapter also identifies the various interacting causes underpinning this diversity in inter-individual response, and, finally, proposes potential research questions that, if answered, will facilitate the evolution of more personalised guidelines for caffeine use within sporting contexts.

2. Inter-subject variation in the response to caffeine

Whilst caffeine's ergogenic effects are clear, the research findings demonstrating these benefits are conventionally calculated using the mean cohort responses. Crucially, these mean responses are considered an accurate estimation of the likely responses of each individual within the group. Yet numerous studies over the course of the past two decades illustrate the extent of individual variation commonly occurring subsequent to introduced interventions. The magnitude of this inter-individual response is well demonstrated in studies investigating individual fitness adaptation responses to carefully controlled exercise interventions (Bouchard & Rankinen, 2001; Hubal et al., 2005; Mann et al., 2014). Is this also the case when it comes to the ergogenic effects of caffeine ingestion?

A small number of papers provide some insight into this question, either by directly studying the inter-subject variability in response to caffeine, or by publishing individual subject data. Jenkins et al. (2008) compared the effects of low caffeine doses (1, 2, & 3 mg/kg) against placebo on a 15-minute maximum cycle in 13 cyclists. The main finding was that caffeine improved mean performance by 3.9% (2 mg/kg) and 2.9% (3 mg/kg) respectively versus placebo, with no improvements in the 1 mg/kg trial. This suggests that doses of 2 mg/kg and 3 mg/kg are ergogenic for endurance performance. However, inspection of the individual data demonstrates large inter-individual variation in these effects. Most participants exhibited large variations, with a performance decrement at some doses of caffeine, and performance enhancement at others. One subject, for example, did not demonstrate an ergogenic effect at any dose, whereas four participants found caffeine ergogenic at all doses. Similarly, in a randomised, cross-over trial design, Graham and Spriet (1991) put seven runners through treadmill and cycle ergometer exercise trials to exhaustion with either placebo or 9 mg/kg of caffeine. The caffeine dose significantly improved time to exhaustion for all participants, but there was a large variation in the magnitude of this effect, with performance in the caffeine trial lasting between 105-250% of the length of the placebo trial. Other studies support this variation in ergogenic response to caffeine supplementation, with some individuals showing large improvements, and others no, or even negative, effects of caffeine supplementation (Meyers & Cafarelli, 2005; Vanata et al., 2014; Guest et al., 2018).

3. Why does this individual response exist?

3.1 The genetics of individual variation in caffeine response

As with other complex phenotypes, individual responses following caffeine ingestion are polygenic phenomena, mediated by multiple interacting genes (Bouchard et al., 2011; Timmons 2011). This doesn't mean that it is impossible to determine the genetic drivers of individual differences, however. For example, habitual caffeine use is a highly complex trait, but genome-wide association studies have found single nucleotide polymorphisms (SNPs) associated with this behaviour (Cornelis et al., 2011). Such findings indicate that, whilst genetic differences cannot explain all the variation, they can at least explain some. The below section examines variation within two genes that may impact caffeine ergogenicity, including a discussion regarding the mechanisms underlying this variation.

3.1.1 CYP1A2

The gene *CYP1A2* encodes cytochrome P450 1A2, an enzyme responsible for up to 95% of all caffeine metabolism (Gu et al., 1992). A SNP within this gene, rs762551, affects the speed of caffeine metabolisation. AA homozygotes ("fast" metabolisers) tend to produce more of this enzyme, and therefore metabolise caffeine more quickly. Conversely, C allele carriers ("slow metabolisers") tend to have slower caffeine clearance (Sachse et al., 1999). The effects of this SNP are most well-established in regard to health, with myocardial infarction and hypertension risk increased in slow metabolisers consuming moderate (3-4 cups) amounts of coffee, whilst fast metabolisers exhibit a protective effect of moderate coffee consumption (Cornelis et al., 2006; Palatini et al., 2009).

These earlier medical studies prompted research into how the *CYP1A2* polymorphism might modify the ergogenic effects of caffeine. Womack and colleagues (2012) put thirty-five trained male cyclists through two 40-km cycle time trials, following consumption of either 6 mg/kg of caffeine or placebo 60-minutes beforehand. There was a significant effect of *CYP1A2* genotype on the ergogenic effects of caffeine, with AA genotypes (fast metabolisers; 4.9% improvement) seeing a significantly greater performance improvement than C allele carriers (slow metabolisers; 1.8% improvement). Within AA genotypes, caffeine improved performance by at least one minute for 15 out of 16 participants, whilst in C allele carriers only 10 of 19 participants saw an improvement greater than one minute. These findings allowed the authors to conclude that caffeine has a greater ergogenic effect for *CYP1A2* AA genotypes than C allele carriers.

Since this initial paper, a small number of subsequent studies have been published. The same group published a paper hampered by a lack of CC genotypes, putting 38 recreational cyclists through four 3-km time trials under different experimental conditions; placebo mouth rinse + placebo ingestion, placebo mouth rinse + caffeine ingestion, caffeine mouth rinse + placebo ingestion, and caffeine mouth rinse + caffeine ingestion (Pataky et al., 2015). Both AC (4.1%) and AA (3.4%) genotypes saw performance improvements in the combined caffeine mouth rinse and ingestion trial, but only AC (6%) genotypes saw a performance improvement in the caffeine ingestion trial. The conclusion was that AC genotypes saw greater performance enhancement with caffeine ingestion, in contrast to Womack and colleagues (2012). One potential confounder identified by Pataky and colleagues (2015) was the shorter exercise trial duration (~5 minutes) when compared to Womack et al. (2012). A second potential confounder is that Womack and colleagues (2012) utilised trained participants, whilst Pataky et al. (2015) did not. Exercise appears to increase *CYP1A2* expression (Vistisen et al., 1992; Kochanska-Dziurowicz et al., 2015), such that trained and untrained individuals may metabolise caffeine differently. Algrain et al. (2006) reported no modifying effect of the *CYP1A2* polymorphism on the ergogenic effects of caffeine; however, they noted the small subject number (n=20), the untrained status of these participants, and the lower caffeine dose (approximately 255 mg). Klein et al. (2012) and Salinero et al. (2017) found no effect of the *CYP1A2* polymorphism on the effects of caffeine on tennis and Wingate test performance respectively, although with modest sample sizes (n=16 and 21).

More recent studies have been able to add some clarity to the potential impact of this polymorphism with *CYP1A2* on the ergogenic effects on caffeine. In a study with by far the largest sample size yet, Guest and colleagues (2018) put 101 trained male participants through three 10-km cycle ergometer time trials with placebo, 2 mg/kg caffeine or 4 mg/kg caffeine. Whilst these caffeine doses exerted ergogenic effects in AA genotypes, leading to a 4.8% and 6.8% improvement at the 2 and 4 mg/kg doses respectively, the ergogenic effects were not present in C allele carriers. The AC genotypes exhibited no performance improvements following caffeine ingestion, whilst for CC genotypes the 4 mg/kg dose lead to a 13.7% increase in time trial performance, representing a significantly ($p=0.04$) ergolytic effect. Similarly, Rahimi (2018) recruited 30 resistance trained males to undertake two resistance training sessions, one with placebo and one with 6 mg/kg caffeine, in a randomised cross-over study design. For A allele carriers, the participants were able to carry out a greater number of repetitions following caffeine consumption, whilst caffeine had no such impact on C allele carriers.

Finally, whilst this polymorphism may well have positive effects on physical performance, it's not yet clear whether these improvements enhance team sport performance. Kingsley et al. (2017) examined the interaction of caffeine (3 mg/kg) and *CYP1A2* genotype on a simulated soccer game, specifically exploring differences in sprint performance. Whilst individual differences in caffeine response were evident, *CYP1A2* genotype did not explain this variation; potentially due to a lack of statistical power on account of the low subject numbers (n=10). Similarly, Puente et al., (2018) recruited 19 elite male (n=10) and female basketball players and subjected them to a vertical jump and agility test, as well as a simulated basketball game, with no difference between the genotype groups in terms of match performance in the caffeine trial (dose = 3 mg/kg), although the caffeine itself was ergogenic.

At present, the initial Womack et al. (2012) paper has only recently been satisfactory replicated (Guest et al., 2012), with some subsequent published research finding no impact of the *CYP1A2* polymorphism (Algrain et al., 2016), or the opposite effect (Pataky et al., 2015). Many of these subsequent papers have, however, tended to employ small sample sizes, in untrained individuals, or void of CC genotypes, present in approximately 10% of the population (Sachse et al., 1999). Further work is required to determine the full effect of this polymorphism on the ergogenic effects of caffeine on exercise, and whether knowledge of *CYP1A2* genotype can enhance performance.

3.1.2 *ADORA2A*

A SNP in the adenosine receptor gene *ADORA2A*, rs5751876, affects both habitual caffeine use (Cornelis et al., 2007) and sleep disturbances following caffeine use (Retey et al., 2007; Byrne et al., 2012). Currently, only one pilot study has examined the effect of this SNP on the ergogenic effects of caffeine (Loy et al., 2015). Twelve female participants underwent a randomised, double-blinded crossover trial comprised of two 10-minute time trials following caffeine ingestion (5 mg/kg) or placebo. The TT homozygotes found caffeine ergogenic; the C allele carriers tended not to, with only one out of the six C allele carriers exhibiting an ergogenic effect. These participants habitually consumed no caffeine or only low doses of caffeine (<250 mg/day), so it's not apparent how this might affect users

habituated to higher doses. Subsequent research is required to replicate these findings, including within habitual caffeine users.

Single Nucleotide Polymorphism	Study	Design	Sample Characteristics	Caffeine Dose	Measurement	Primary Outcome
CYP1A2 (rs762551)	Womack et al. (2012).	Caffeine vs placebo	36 male recreationally competitive cyclists	6 mg/kg, 60 minutes prior.	40km cycle time trial	Caffeine reduced 40km time trial time vs placebo by a greater ($p<0.05$) magnitude in AA vs C allele carriers.
	Klein et al. (2012).	Caffeine vs placebo	16 Collegiate male (n = 8) and female (n = 8) tennis players	6 mg/kg, 60 minutes prior	Maximal treadmill exercise test, tennis skills test.	No significant impact of polymorphism on caffeine ergogenicity.
	Pataky et al. (2015).	Caffeine ingestion, placebo ingestion, caffeine mouth rinse, placebo mouth rinse.	30 male (n = 25) and female (n = 13) recreational cyclists	6 mg/kg, 60 mins prior, along with 25 mL of 1.14% caffeine mouth rinse	3km cycle time trial.	Greater performance enhancement in AC vs AA in both caffeine ingestion and caffeine rinse trials (no CC genotypes present).
	Algrain et al. (2016).	Caffeine gum vs placebo	20 recreationally active males (n = 13) and females (n = 7)	300 mg caffeine gum, 10 minutes prior.	15-minute steady state cycle, 10 minutes recovery, 15 minute performance ride at 75% VO_{2max}.	No significant impact of polymorphism on caffeine ergogenicity.
	Salinero et al. (2017).	Caffeine vs placebo	21 recreationally active males (n	3 mg/kg	30 s Wingate Test.	No significant impact of polymorphism

			= 14) and females (n = 7)			on caffeine ergogenicity.
	Guest et al., (2018).	Caffeine vs placebo	101 active males	2 mg/kg & 4 mg/kg	10km cycle ergometer TT.	Caffeine reduced performance at 4 mg/kg for CC genotypes, but increased performance for AA genotypes.
	Rahimi (2018).	Caffeine vs placebo	30 resistance trained males.	6 mg/kg	3 sets to failure over 5 exercises.	AA genotypes performed a greater number of repetitions with caffeine vs placebo; C allele carriers did not.
	Puente et al., (2018).	Caffeine vs placebo	19 (male = 10) elite basketball players.	3 mg/kg	Vertical jump, agility test, simulated match.	AA genotype had a performance enhancement in the vertical jump test with caffeine, whilst C allele carriers did not. There were no genotype differences in the other tests.
ADORA2A (rs5751876)	Loy et al. (2015).	Caffeine vs placebo	12 females	5 mg/kg	20 min cycle at 60% VO_{2max}, followed by 10 minute maximum cycle.	Total work increased for time trial genotypes following caffeine ingestion vs placebo. There were no improvements in the caffeine

	vs placebo trial for C allele carriers.

Table 1 – Summary of published studies examining *CYP1A2* and *ADORA2A* polymorphisms and the ergogenic effect of caffeine on performance.

3.1.3 Potential mechanisms – A role for caffeine timing?

It is clear that genetic factors exert a large influence on individual responses to caffeine ingestion, even if these genetic factors have not yet been well elucidated. The mechanisms through which this genetic variation modifies caffeine ergogenicity are also unclear; regarding *CYP1A2,* it is speculated it could be due to a more rapid accumulation of caffeine metabolites in AA genotypes, which are hypothesised to potentially have a greater ergogenic effect than caffeine itself (Womack et al., 2012). The mechanism proposed by Guest et al. (2018) is that, because C allele carriers metabolise caffeine at a slower rate than AA genotypes, they experience prolonged vasoconstriction, which is likely to be performance limiting in endurance events where the transfer of oxygen and nutrients to the working muscle is crucial. If either, or both, of these mechanisms are correct, then caffeine timing becomes important; it might not be that C allele carriers find caffeine less ergogenic, just that it requires longer for caffeine to be metabolised to its ergogenic metabolites. Given caffeine's many different mechanisms of action, it's likely each mechanism has polymorphisms that modify the ergogenic effects. For example, as caffeine reduces exercise induced pain (Gonglach et al., 2016), SNPs related to pain tolerance could modify this effect. Similarly, genetic variation in adenosine receptors (such as polymorphisms within *ADORA2A*) are similarly promising. In the pilot study carried out by Loy et al. (2015) there were a number of mechanisms proposed by the authors through which *ADORA2A* variation might affect caffeine ergogenicity, including enhanced motivation and motor unit recruitment in TT homozygotes.

3.1.4 Indirect impact of genetic variation on exercise performance

Genetic variation also likely impacts exercise performance indirectly. Thomas et al. (2016) examined the modifying effects of the *CYP1A2* polymorphism on recovery from exercise. Whilst overall there was no effect of the polymorphism on cardiac markers of recovery, there were significant differences in the square root of the mean of squared differences between successive R intervals (RMSSD) in heart rate variability monitoring. Similarly, polymorphisms within *ADORA2A* can predispose individuals to increased anxiety following caffeine ingestion (Alsene et al., 2003; Rogers et al., 2010). This is potentially of interest in individuals who suffer from pre- and within-competition anxiety, but also to individuals who may benefit from elevated levels of pre-competition arousal. *ADORA2A* polymorphisms are also associated with increased sleep disturbances following caffeine ingestion (Retey et al., 2007), which could affect individuals involved in evening competitions, or those involved in tightly spaced consecutive day competitions; here, sleep disturbances could negatively affect exercise recovery.

3.2 Environmental factors affecting caffeine response

There are also a variety of different non-genetic factors that can affect caffeine ergogenicity, many of which are often controlled for in research. These include habitual use of caffeine, with habitual use assumed to potentially reduce the ergogenic effect of caffeine (Bangsbo et al., 1992; Bell & McLellan, 2002; Beaumont et al., 2016), although this finding is equivocal (Irwin et al., 2011; Goncalves et al., 2017); perhaps habitual users simply require higher doses of caffeine to maintain the ergogenic effect. Other non-genetic factors affect caffeine metabolisation speed, often by increasing cytochrome P450 activity. These include smoking (Parsons & Neims, 1978; Schrenk et al., 1998), dietary vegetable intake (Lampe et al., 2000), oral contraceptive use (Rietveld et al., 1984; Abernethy & Todd, 1985), pregnancy (Knutti et al., 1981), menstrual cycle stage (Lane et al., 1992), training status (Vistisen et al., 1992; Kochanska-Dziurowicz et al., 2015) and hormone replacement therapy (Pollock et al., 1999). Other non-genetic, but controllable, factors affecting caffeine ergogenicity are related to the nature of caffeine ingestion, including caffeine dose (Graham & Spriet, 1995), source (Graham et al., 1998; Hodgson et al., 2013; Higgins et al., 2016), age (Tallis et al., 2017), timing (Boyett et al., 2016), time of day (Mora-Rodriguez et al., 2015, Boyett et al., 2016) and training status (LeBlanc et al., 1985; Collomp et al., 1992).

Finally, expectancy effects influence caffeine response. Saunders et al. (2016) put participants through time trials with either 6 mg/kg of caffeine, placebo or control (neither caffeine nor placebo). Correct identification of caffeine ingestion gave a greater relative performance enhancement than the overall caffeine trial. Similarly, the belief that caffeine had been ingested in the placebo trial lead to a *likely beneficial* effect, quantified via the magnitude-based inferences method. Correct identification of placebo lead to *possibly harmful* effects, with some participants showing a performance decrement compared to the control trial. This mirrors results of earlier research on expectancy effects and caffeine. For example, Beedie et al. (2006) showed that placebo caffeine ingestion improved endurance cycle performance in a dose-response manner, with higher placebo doses leading to greater performance improvements. Similarly, Pollo et al. (2008) demonstrated that belief of caffeine ingestion improved time to fatigue in a maximal quadriceps extension task. When participants are informed they have ingested caffeine, it appears to improve performance, even if they have been deceptively administered a placebo (Foad et al., 2008; Saunders et al., 2016).

It is also important to consider that genetics also modify these environmental factors. For example, habitual caffeine use itself has a genetic underpinning (Josse et al., 2012), and certain genotypes appear to be more sensitive to the effects of placebo (Hall et al., 2015).

3.3 Epigenetic modifiers of caffeine response

Epigenetics refers to changes in gene function that occur without a change in nucleotide sequence (Ling & Groop, 2009). Such changes can be heritable, but also modifiable over time within an individual (Moran & Pitsiladis, 2016). Caffeine use undoubtedly induces epigenetic modifications (Buscariollo et al., 2014; Ping et al., 2014; Wendler et al., 2014), and these epigenetic modifications can impact caffeine clearance by altering CYP1A2 activity (Hammons et al., 2001; Jin et al., 2004). However, it is not entirely clear how this might alter caffeine's ergogenic effects. Long-term caffeine use potentially leads to habituation through both increased caffeine clearance—mediated by epigenetic modifications on cytochrome P450 genes (Hammons et al., 2001)—and a decrease of excitability caused by caffeine, possibly via inhibition of genes affecting the dopaminergic and adenosine pathways (Van Soeren et al., 1993). Further research is required to establish the effects of epigenetics on the ergogenic effects of caffeine.

3.4 "Non-responder" vs "Did not respond"

Clearly, the individual response to caffeine is complex, and subject to genetic, non-genetic (i.e. environmental), and epigenetic influence. Given that both environmental and epigenetic influences are not stable across time, an individual's response to caffeine will vary. A clear example of this is that of habituation, briefly discussed in section 3.2. In this context, regular use of caffeine may modify the ergogenic effects of caffeine at a particular dose. Beaumont et al. (2016) illustrated that regular intakes of 3 mg/kg of caffeine daily attenuated the ergogenic effects of a pre-exercise dose of 3 mg/kg. Conversely, Goncalves et al. (2017) showed that habitual daily caffeine intakes of 350 mg/day were insufficient to reduce the ergogenic effects of 6 mg/kg of caffeine. This indicates that it is perhaps important that the pre-exercise caffeine dose exceeds the level of habitual intakes. So, whilst an individual might initially find a caffeine dose of 3 mg/kg ergogenic, if they then habitually consume 3 mg/kg of caffeine per day, this ergogenisis may be attenuated. As such, in an initial trial, the subject would be labelled as a caffeine "responder", whilst in the subsequent trial, they would be labelled a "non-responder". Such labels are becoming commonplace when reporting on inter-individual response to a stimulus. However, recent work (Montero & Lundby, 2017) indicates that non-response to exercise can be reduced by changing training variables. As discussed in depth in Chapter 3, based on the available research, it appears likely that the same is true for caffeine. As such, perhaps a more reflective characterisation would be to state that a subject "did not respond" to a particular intervention, as opposed to labelling them a "non-responder" (Betts & Gonzalez, 2016; Pickering & Kiely, 2018b), as this non-response may not occur were the intervention to be repeated and/or modified.

4. Conclusions – what next?

Academic studies have repeatedly demonstrated a performance enhancing effect of caffeine ingestion (Graham 2001; Burke 2008; Glaister et al., 2008; Astorino & Roberson, 2010). Yet, simultaneously, this ergogenic response shows considerable inter-individual variation (Graham & Spriet, 1991; Jenkins et al., 2008). This variation occurs via numerous factors, many of which are influenced by genetic predispositions (Womack et al., 2012; Loy et al., 2015). Although these individual responses are undoubtedly complex and subject to various modifying factors, the possibility remains that practitioners can glean sufficient partial insights to personalise caffeine intake. Polymorphisms in genes affecting caffeine metabolisation speed (*CYP1A2)* (Womack et al., 2012; Guest et al., 2018) and nervous system excitability *(ADORA2A)* (Loy et al., 2015) appear to directly modify the ergogenic effects of caffeine. Given the number of mechanisms through which caffeine appears to exert its action, it could be speculated that a variety of other polymorphisms will also have a contributing role. Recent developments in genetic profiling technology and more widespread access to, and affordability of, such technology raises the possibility that such insights may soon be readily available to sporting populations. This information could potentially be paired with knowledge of individual variation in other factors, such as circadian rhythm (Mora-Rodriguez et al., 2015; Boyett et al., 2016), habitual caffeine use (Bangsbo et al., 1992; Bell & McLellan, 2002; Beaumont et al., 2016), medication intake (Rietveld et al., 1984; Abernethy & Todd, 1985), and expectancy (Beedie et al., 2006; Pollo et al., 2008; Saunders et al., 2016), all of which also affect the magnitude of performance enhancement seen after caffeine ingestion.

These individualised caffeine guidelines could also vary depending on the timing and importance of the competition. Given that genetic variation can modify sleep disturbances after caffeine ingestion (Retey et al., 2007), individuals more likely to suffer from these disturbances might consume less caffeine for an evening competition than a morning competition. This would be especially important if there were a number of competitions in close proximity, whereby reduced recovery following initial caffeine dose may impact subsequent exercise performance. Genetic variation can also impact feelings of anxiety following caffeine ingestion (Alsene et al., 2003; Rogers et al., 2010). This creates the possibility that certain genotypes should consume less caffeine for competitions where anxiety is likely to be higher, such as the Olympic Games or World Cup final, and more for competitions where anxiety will be lower, such as a league match. Figure 5 below details some of the potential recommendations that could be made based on an individual's genotype in the future.

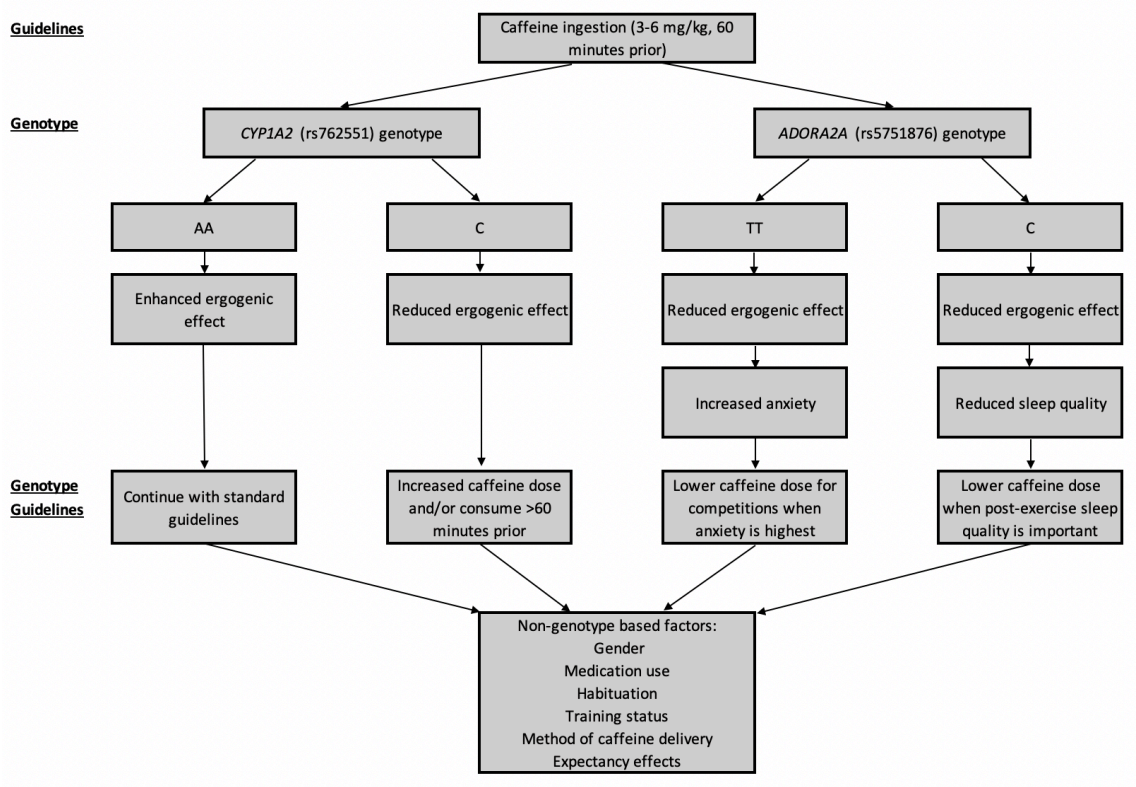

Figure 5 – Genetic and non-genetic factors influencing caffeine ingestion decisions. Working from the top, the current best-practice guidelines are applied to different genotypes of genes identified to impact caffeine response. Based on the current evidence, genotype-based guidelines are then produced. Finally, these genotype guidelines must then be interpreted in the context of non-genetic factors, such as habitual use, to create individualised caffeine guidelines. As *CYP1A2* and *ADORA2A* polymorphisms haven't yet been studied together, the potential interacting effects of these polymorphisms are currently unknown. Finally, the recommendations themselves are somewhat speculative, and further research is required to elucidate best practice in this area.

The above discussion drives an interesting situation; whilst caffeine is ergogenic, the current generalised guidelines of 3-9 mg/kg, 60-mins prior (Ganio et al., 2009; Goldstein et al., 2010b; Brooks et al., 2016) are clearly not optimal for everyone. What is not clear, however, is what these guidelines should be. Being able to develop more precise, individualised guidelines would be beneficial, especially given the prevalent use of caffeine in elite sports. To enhance the advice given to athletes regarding caffeine use, a number of different questions will need to be answered:

1. Can the existing research on *CYP1A2* and *ADORA2A* be replicated, and can other genes that modify caffeine ergogenicity be identified?
2. Are there different optimal dosages and timing strategies for different genotypes?
3. Does caffeine habituation occur differently across genotypes?
4. Does the individual's sex further alter the modifying aspect of genotype on caffeine ergogenicity?

Furthermore, if the proposed mechanisms regarding how the *CYP1A2* polymorphism affects caffeine ergogenicity are indeed correct (section 3.1.3), then there remains the possibility that caffeine can still be ergogenic for C allele carriers, but that such individuals need to consume it a greater amount of time prior to exercise. In the majority of studies exploring the ergogenic effects of caffeine, it is consumed ~60 minutes pre-exercise. However, for C allele carriers, could the ergogenic effects of caffeine be restored by utilising a caffeine dose 90- or 120-minutes pre-exercise? Such a hypothesis is, of course, speculative, and requires testing—but it does represent a potential way by which caffeine can indeed be ergogenic for all. The resolution of whether caffeine is truly ergolytic or neutral for *CYP1A2* C allele carriers, or if it merely necessitates a different caffeine strategy, represents an important step on the journey towards more personalised sports nutrition guidelines. By answering this, and the above, questions and creating personalised caffeine guidelines, athletes will be able to fully maximise the performance enhancing effects of caffeine in a way that is matched to their unique biology. In addition, the awareness from coaches and athletes that sizeable variation exists in the response to caffeine ingestion may encourage them to be more experimental and flexible in the evolution of their caffeine strategies.

CHAPTER 6 – *ACTN3*: MORE THAN JUST A GENE FOR SPEED

Chapter preface:

ACTN3 is the most well-research gene in terms of sports performance, and a common SNP within it is strongly associated with elite speed-power status. As such, this gene has often been referred to as a "gene for speed" (MacArthur & North, 2004; Chan 2008; Berman & North 2010). However, recent research suggests that this SNP has the potential to modify other aspects of performance, such as exercise adaptation, post-exercise recovery, and injury risk. As such, the purpose of this chapter is to present evidence of a modifying effect of *ACTN3* on these dimensions of sports performance, suggesting that knowledge of *ACTN3* genotype might be useful within a sporting setting. This chapter was published as a paper in *Frontiers in Physiology* (Pickering & Kiely, 2017d), and was the first review article to explore *ACTN3* beyond the realm of its association with elite athlete status.

1. Introduction

ACTN3 is a gene that encodes for α-actinin-3, a protein expressed only in type-II muscle fibres (North et al., 1999). A common polymorphism in this gene is R577X (rs1815739), where a C-to-T base substitution results in the transformation of an arginine base (R) to a premature stop codon (X). X allele homozygotes are deficient in the α-actinin-3 protein, which is associated with a lower fast-twitch fibre percentage (Vincent et al., 2007), but does not result in disease (MacArthur & North, 2004). The XX genotype frequency differs across ethnic groups, with approximately 25% of Asians, 18% of Caucasians, 11% of Ethiopians, 3% of Jamaican and US African Americans, and 1% of Kenyans and Nigerians possessing the XX genotype (Yang et al., 2007; MacArthur et al., 2008; Scott et al., 2010). *ACTN3* genotype is associated with speed and power phenotypes. Yang et al. (2003) reported that elite sprint athletes had significantly higher frequencies of the R allele than controls, a finding that has been replicated multiple times in speed, power, and strength athletes (Druzhevskaya et al., 2008; Roth et al., 2008; Eynon et al., 2009c; Ahmetov et al., 2011; Cieszczyk et al., 2011; Kikuchi et al., 2016; Papadimitriou et al., 2016; Weyerstraß et al., 2017; Yang et al., 2017), although these findings are not unequivocal (Gineviciene et al., 2001; Scott et al., 2010; Sessa et al., 2011). Whilst Yang et al. (2003) found a trend towards an increased XX genotype frequency in endurance athletes versus controls, this relationship is less robust, with most studies reporting a lack of association between XX genotype and endurance performance (Lucia et al., 2006; Saunders et al., 2007; Doring et al., 2010b; Kikuchi et al., 2016). In addition, whilst Kenyan and Ethiopian endurance runners are highly successful (Wilber & Pitsiladis, 2012), the frequency of the XX genotype within this group is very low at 8% (Ethiopian) and 1% (Kenyan) (Yang et al., 2007). As such, the general consensus is that *ACTN3* X allele likely does not modify elite endurance athlete status (Vancini et al., 2014).

Much of the attention on *ACTN3* has focused on the robust relationship with the R allele and strength/power phenotype, with a number of reviews further exploring this relationship (Eynon et al., 2013; Ma et al., 2013; Ahmetov & Fedotovskaya 2015). Indeed, a number of papers have referenced

ACTN3 as a "gene for speed" (MacArthur & North, 2004; Chan 2008; Berman & North 2010). However, emerging evidence suggests that this polymorphism may influence a number of other traits, including exercise recovery, injury risk, and training adaptation (Delmonico et al., 2007; Pimenta et al., 2012; Massidda et al., 2017). The purpose of this chapter is to further explore these potential relationships, as an increased understanding of the role played by *ACTN3* on these traits may lead to improvements in the utilisation of genetic information in exercise training.

2. *ACTN3* as a modulator of training response

Over the last twenty or so years, the consistent underlying impact of genetics on exercise adaptation has been well explored (Bouchard et al., 2011; Bouchard 2012). Whilst it is clear that genetic variation has an undoubted influence on both exercise performance (Guth & Roth, 2013) and adaptation (Mann et al., 2014), fewer studies examine the influence of individual single nucleotide polymorphisms (SNPs) (Delmonico et al., 2007), or a combination of SNPs (Jones et al., 2016), on this process. This sub-section explores the evidence regarding the impact of *ACTN3* on the post-exercise adaptive response.

Following a structured literature search, five studies examining the influence of *ACTN3* on exercise adaptation to a standardised training programme were found (table 2). Four of these studied resistance training (Clarkson et al., 2005b; Delmonico et al., 2007; Pereira et al., 2013; Erskine et al., 2014), and one focused on aerobic training (Silva et al., 2015). An additional study (Magi et al., 2016), monitored changes in VO_{2peak} over a five-year period in elite skiers, with no significant *ACTN3* genotype differences. However, the exercise intervention in this study was not controlled (i.e. participants were undertaking differing training programmes in a real-world setting), and so it is not included within table 2. There was considerable variation in the findings. For resistance training, two studies reported that the RR genotype was associated with the greatest increase in strength (Pereira et al., 2013) and power (Delmonico et al., 2007) following resistance training. One study reported no effect of *ACTN3* genotype on training adaptations following resistance training (Erskine et al., 2014). Another reported greater improvement in one-repetition maximum (1RM) strength in X allele carriers compared to RR genotypes (Clarkson et al., 2005b). A further study utilised *ACTN3* within a 15-SNP total genotype score (TGS), finding that individuals with a higher number of power alleles (such as *ACTN3* R) exhibited greater improvements following high-intensity resistance training compared to low-intensity resistance training (Jones et al., 2016). However, because participants could have the *ACTN3* XX genotype and still be classed as those who would best respond to high-intensity training (due to the possession of a higher number of alleles in other power-associated SNPs), this study is not included within table 2.

Study	Method	Sample Characteristics	Main Outcome
Clarkson et al. (2005b)	12 weeks progressive resistance exercise training on non-dominant arm. Progression from 3 sets of 12 repetitions to 3 sets of 6 repetitions, with concurrent increase in load.	602 (355 females) aged 18-40 (n=133 XX genotype.	In females, the X allele was associated with greater absolute and relative improvements in 1RM vs RR genotypes.
Pereira et al. (2013)	12-week high-speed power training programme. Progression from 3 sets of 10 repetitions @ 40% 1RM to 3 sets of 4 repetitions @ 75% 1RM.	139 Older (mean = 65.5y) Caucasian females (n=54 XX genotype).	RR genotypes exhibited greater performance improvements (maximal strength, CMJ) compared to X allele carriers.
Erskine et al. (2014)	9-week unilateral knee extension resistance training programme.	51 previously untrained young males (n=7 XX genotype).	Responses to resistance training were independent of *ACTN3* genotype.
Silva et al. (2015)	18-week (3 sessions per week) endurance training programme, comprised primarily of 60-minutes running, individually controlled by heart rate monitor use.	206 male Police recruits (n=33 XX genotype).	At baseline, XX genotypes had greater VO_2 measure scores than RR genotypes. Following training, this difference disappeared; i.e. RR had greater improvements than XX.
Delmonico et al. (2007)	10-week (3 session per week) unilateral knee extensor strength training comprised of 4-5 sets of 10 repetitions.	155 (n=86 females) older (50-85y) participants (n=39 XX genotype).	Change in absolute peak power greater in RR vs XX (p=0.07) for males. Relative peak power change greater in RR vs XX (p=0.02).

Table 2 – Studies examining the interaction between *ACTN3* genotype and exercise adaptation.

The variation between studies is likely due to heterogeneity at baseline between genotypes, and differences in exercise prescription. Given the prevalence of the R allele in elite speed-power and strength athletes (Yang et al., 2003, Vincent et al., 2007), it is speculatively considered that R allele carriers would respond best to speed-power and strength training (Kikuchi & Nakazato 2015). However, as illustrated here, there is perhaps a paucity of data to support this position. Nevertheless, there are some potential molecular mechanisms that could underpin this proposition. Norman et al. (2014) reported that mammalian target of rapamycin (mTOR) and p70S6k phosphorylation was greater in R allele carriers than XX genotypes following sprint exercise. Both mTOR and p70S6k regulate skeletal muscle hypertrophy (Bodine et al., 2001; Song et al., 2005), providing mechanistic support for the belief that hypertrophy, and hence strength and power improvements, should be greater in R allele carriers following resistance training. In addition, Ahmetov and colleagues (2014a) reported that testosterone levels were higher in male and female athletes with at least one R allele compared to XX genotypes. Whilst the direction of this association is not clear, it again supplies a possible mechanism explaining why R allele carriers may experience greater training-induced strength improvements.

A single study examined the impact of this polymorphism on the magnitude of VO_2 improvements following endurance training (Silva et al., 2015). Here, VO_2 scores at baseline were greater in XX genotypes, but following training this difference was eliminated, indicating that RR genotypes had a greater percentage improvement following training. The population in this cohort were police recruits. Given that the X allele is potentially associated with elite endurance athlete status (Yang et al., 2013), it is not clear whether these results would be mirrored in elite endurance athletes. Clearly, further work is required to fully understand what relationship, if any, exists between *ACTN3* and improvements in aerobic capacity following training.

3. *ACTN3* as a modulator of post-exercise recovery

ACTN3 R577X has also been associated with exercise-induced muscle damage; here, increased muscle damage will likely reduce speed of recovery, suggesting a potential modifying effect of this polymorphism on between-session recovery. Of the eight studies identified that examined the impact of this polymorphism on post-exercise muscle damage (table 3), six reported that that the X allele and/or the XX genotype was associated with higher levels of markers associated with muscle damage (Vincent et al., 2010; Djarova et al., 2011; Pimenta et al., 2012; Belli et al., 2017; Del Coso et al., 2017b; Del Coso et al., 2017c). One study found no effect of the polymorphism (Clarkson et al., 2005a), and one found that RR genotypes experienced a greater exercise-induced reduction in force compared to XX genotypes (Venckunas et al., 2012). An additional investigation (Del Coso et al., 2017a) examined the impact of *ACTN3* as part of a TGS on creatine kinase (CK) response following a marathon race. Within this TGS, the R allele was considered protective against increased CK concentrations. The results indicated that those athletes with a higher TGS, and therefore greater genetic protection, had a lower CK response to the marathon. Whilst not direct evidence of the R allele's protective effect, as it is possible that the other SNPs used in the TGS conveyed this effect, it nevertheless strengthens the supporting argument.

Study	Method	Sample Characteristics	Main Outcome
Pimenta et al. (2012)	Eccentric-contraction based training session	37 male professional soccer players based in Brazil. (n=9 XX genotype)	Greater creatine kinase (CK) activity in XX genotypes vs RR
Clarkson et al. (2005a)	50 maximal eccentric contractions of the elbow flexor	157 male (n=78) and female participants of various ethnicities (n=115 Caucasians; n=48 XX genotype)	No association of R577X with increases in CK and myoglobin (Mb) following eccentric exercise.
Vincent et al. (2010)	4 x 20 maximal single leg eccentric knee extensions	19 healthy young males (n=10 XX genotype)	XX genotypes had greater peak CK activity post-training compared to RR genotypes, and reported greater increases in muscle pain.
Venckunas et al. (2012)	Two bouts of 50 drop jumps	18 young males (n=9 XX genotype)	RR showed greatest decrease in voluntary force, and slower recovery, compared to XX genotypes.
Djarova et al. (2011)	Resting blood sample	31 South African Zulu males (n=14 Cricketers and n=17 controls). No XX genotypes.	R allele associated with lower CK levels (RR vs RX)
Del Coso et al. (2017b).	Marathon race, pre- & post-race Counter Movement Jump (CMJ).	71 experienced runners (n=8 XX genotype).	X allele carriers had higher CK and Mb levels post-race compared to RR homozygotes. X allele carriers also had a greater reduction in leg muscle power compared to RR genotypes.
Del Coso et al. (2017c).	Triathlon competition (1.9km swim, 75km cycle, 21.1km run), pre- & post-race CMJ.	23 healthy, experienced triathletes (n=19 males, n=5 XX genotype).	X allele carriers had a more pronounced jump height reduction compared to RR

			genotypes. In X allele carriers, there was a tendency towards higher post-race Mb concentrations (P = 0.10) and CK concentrations (P = 0.06) compared to RR homozygotes.
Belli et al. (2017)	37.1km adventure race (22.1km mountain biking, 10.9km trekking, 4.1km water trekking, 30m rope course).	20 well trained athletes (n=15 males; n=4 XX genotype).	XX genotypes had higher concentrations of serum Mb, CK, lactate dehydrogenase (LDH) and AST compared to R allele carriers.

Table 3 – Studies examining the interaction between *ACTN3* genotype and exercise recovery

The increase in post-exercise muscle damage in X allele carriers is likely due to structural changes associated with this polymorphism. Alpha-actinin-3 is expressed only in fast-twitch muscle fibres, and X allele homozygotes are α-actinin-3 deficient; instead, they upregulate production of α-actinin-2 in these fast-twitch fibres (MacArthur et al., 2007; Seto et al., 2011a). Both α-actinin-3 (encoded for by *ACTN3*) and α-actinin-2 are major structural components of the Z-disks within muscle fibres (Beggs et al., 1992). The Z-disk itself is vulnerable to injury during eccentric contractions (Friden & Lieber 2001), and knock-out mouse models illustrate these Z-disks are less stable during contraction with increased α-actinin-2 concentrations (Seto et al., 2011a). A number of the studies in table 3 exclusively utilised eccentric contractions, whilst others focused on prolonged endurance events that include running, which incorporates eccentric contractions as part of the stretch shortening cycle with each stride (Komi 2000).

The overall consensus of these studies is that the X allele, and/or the XX genotype, is associated with greater markers of muscle damage following exercise that has an eccentric component; either through direct eccentric muscle action (Vincent et al., 2010), from sport-specific training (Pimenta et al., 2012), or from a competitive event requiring eccentric contractions (Del Coso et al., 2017b & 2017c, Belli et al., 2017). However, there are a number of weaknesses to these studies, potentially limiting the strength of these findings. The overall subject number is modest, with a total of 376 (mean 47) across all eight studies; indeed, the study with the greatest number of participants, Clarkson et al. (2005a), reported no modifying effect of this polymorphism on post-exercise muscle damage. The total number of XX genotypes was also low, with 85 reported across the studies. This is partly a function of the lower prevalence (~18%) of this genotype, but again the study with the largest number (n=48) of XX genotypes found no effect of this polymorphism (Clarkson et al, 2005a). It is clear that, in order to increase the robustness of this association, further investigations, with greater participant numbers, are required.

4. *ACTN3* as a modulator of exercise-associated injury risk

Six studies were found to examine the association between *ACTN3* genotype and sports injury prevalence (table 4). Three of these examined ankle sprains (Kim et al, 2014; Shang et al., 2015; Qi et al., 2016), with one each for non-contact injuries (Iwao-Koizumi et al., 2014), professional soccer players (Massidda et al., 2017), and exertional rhabdomyolysis (ER) (Deuster et al., 2013). Whilst ER is strongly related to increased CK following exercise (Clarkson & Ebbeling 1988; Brancaccio et al., 2010), because it requires medical treatment it was classified as an injury, and hence papers exploring ER are included here. Of these papers, five reported a protective effect of the R allele and/or the RR genotype against injury (Deuster et al., 2013; Kim et al., 2014; Shang et al., 2015; Qi et al. 2016; Massida et al., 2017). Specifically, Deuster and colleagues (2013) found that XX genotypes were almost three times more likely to be ER patients than R allele carriers. Qi et al. (2016) reported a significantly lower frequency of the RR genotype in a group of ankle sprain patients versus controls. Kim and colleagues (2014) found that XX genotypes were 4.7 times more likely to suffer an ankle injury than R allele carriers in their cohort of ballerinas. Shang et al. (2015) reported the R allele as significantly under-represented in a cohort of military recruits reporting ankle sprains. Finally, Massidda and colleagues (2017) demonstrated that XX genotypes were 2.6 times more likely to suffer a muscular injury than RR genotypes, and that these injuries were more likely to be of increased severity. Only one study (Iwao-Koizumi et al. 2014) reported that the R allele was associated with an increased risk (OR = 2.52) of a muscle injury compared to X allele carriers in a female cohort.

Study	Method	Sample Characteristics	Main Outcome
Iwao-Koizumi et al. (2014)	Sports injury data survey	99 female students (n=34 XX genotype)	R allele associated with an increased odds ratio (OR) of 2.52 of muscle injury compared to X allele.
Deuster et al. (2013)	Controls – lower body exercise test. Cases – anonymous blood or tissue sample collected after an exertional rhabdomyolysis (ER) incident.	134 controls and 47 ER patients (n=38 XX genotype)	XX genotypes 2.97 times more likely to be to ER cases compared to R allele carriers.
Qi et al. (2016)	Ankle sprain case-control analysis	100 patients with non-acute ankle sprain vs 100 healthy controls (n=89 XX genotype)	Significantly lower frequency of RR genotype in ankle sprain group compared to controls (p = 0.001).
Kim et al. (2014)	Ankle injury case-control analysis.	97 elite ballerinas and 203 normal female adults (n=65 XX genotype)	XX genotypes 4.7 times more likely to suffer an ankle injury than R allele carriers.
Shang et al. (2015)	Ankle injury case-control analysis.	142 non-acute ankle sprain patients and 280 physically active controls (n=87 XX genotype). All military recruits.	RR genotype and R allele significantly under-represented in the acute ankle injury group.
Massidda et al. (2017)	Case control, genotype-phenotype association study	257 male professional Italian soccer players and 265 non-athletic controls.	XX players were 2.6 times more likely to suffer a sports injury than RR genotypes. Severe injuries were also more likely in X allele carriers compared to RR genotypes.

Table 4 – Studies examining the interaction between *ACTN3* genotype and sports injury.

Regarding ER, the likely mechanism is similar to that discussed in the post-exercise muscle damage section; increased damage at the Z-disk during exercise. For ankle sprains, the mechanism is potentially related to muscle function. R allele carriers tend to have greater levels of muscle mass (MacArthur & North, 2007), and specifically type-II fibres (Vincent et al., 2007), indicating that both the RX and RR genotypes tend to have increased strength capabilities (Pimenta et al., 2013). For other soft-

tissue injury types, again, the decreased potential of damage at the Z-disk likely reduces injury risk. This would be particularly true for eccentric contractions; given the importance of this contraction type in the aetiology of hamstring injuries, this could be a further causative mechanism (Askling et al., 2003), alongside that of reduced muscle strength (Yamamoto 1993).

Alongside the modifying role of *ACTN3* on muscle strength and injury risk, emerging evidence suggests this SNP may also affect flexibility and muscle stiffness. Two studies reported an association between the RR genotype and a decreased flexibility score in the sit-and-reach test (Zempo et al., 2016; Kikuchi et al., 2017). Conversely, Kim et al. (2014) reported that XX genotypes had decreased flexibility in the same test. This lack of consensus is largely due to the small total study number, with greater clarity expected as research in the area evolves. It also mirrors the lack of consensus as to whether flexibility increases or decreases the risk of injury (Gleim & McHugh, 1997), indicating the complex, multifactorial nature of injuries and their development (Bahr & Holme, 2003).

In summary, it appears that the R allele of *ACTN3* is somewhat protective against injuries. The mechanisms underpinning this are likely varied, and related to a combination of the modifying effects of this SNP on both strength (particularly eccentric strength), exercise-induced muscle damage, and flexibility.

5. Discussion

The results of this mini-review indicate that, aside from its established role in sporting performance, the *ACTN3* R577X polymorphism also potentially modifies exercise adaption, exercise recovery, and exercise-associated injury risk. As this polymorphism directly influences both muscle structure and muscle fibre phenotype, this is perhaps unsurprising, and points to the potential use of knowledge of this polymorphism in the development of personalised training programmes. However, it is important to consider the limitations surrounding many of these studies. The subject numbers in the considered studies tended to be low, with large heterogeneity between study cohorts, ranging from untrained participants to professional sports people, as well as differences in sex. Both of these aspects likely affect the study findings; the effect of this polymorphism may be smaller in untrained individuals, for example, whereas in elite, well-trained athletes, who are likely closer to their genetic ceiling, the effect may be greater. The low subject numbers are troubling due to the relatively low XX genotype frequency, which is ~18% in Caucasian cohorts, and even lower in African and African-American cohorts. As such, XX genotypes are considerably under-represented across the research.

The above limitations indicate further work is required to fully understand the impact of this polymorphism on these phenotypes. That said, there is some consistency between trials, allowing speculative guidelines to be developed for the use of genetic information in the development of personalised training. XX genotypes potentially have increased muscle damage following exercise that includes an eccentric component (Pimenta et al. 2012, Del Coso et al. 2017b+c, Belli et al. 2017). This information may, consequently, be used to guide between-session recovery, and during the competitive

season, recovery times post-competition. For example, in an elite soccer club, *ACTN3* genotype could be utilised alongside other well-established markers to determine training intensity in the days following a match, with players genetically predisposed to increased muscle damage either having a longer recovery period, or an increased focus on recovery interventions such as cold-water immersion. In addition, recent research has illustrated the positive impact of Nordic Hamstring Exercises on hamstring injury risk (van der Horst et al., 2015), making these exercises increasingly common in professional sports teams. These exercises have a large eccentric component, upon which this polymorphism may have a direct effect. As such, it would be expected that XX genotypes would have increased muscle soreness and damage following these exercises, potentially affecting the timing of their use within a training programme. The potential for genetic information, including that of *ACTN3*, to inform hamstring injury prevention is further explored in Chapter 7.

Focusing on sporting injuries, the general consensus from the studies found is that the X allele increased the risk of ankle injuries (Kim et al., 2014; Shang et al., 2015; Qi et al., 2016) and general sporting injury (Massidda et al., 2017). Again, this information could guide training interventions. In this case, X allele carriers might undertake increased general strengthening exercises and neuromuscular training targeting injury risk reduction. Furthermore, knowledge of this information could increase athlete motivation to undertake these exercises (Goodlin et al., 2015).

Finally, maximising the training response is crucial, both to elite athletes looking to improve by fractions of a second, and to beginners looking to decrease their risk of disease. Increasingly, there is evidence that polymorphisms, including *ACTN3* R577X, can modify this adaptive process (Delmonico et al., 2007; Pereira et al., 2013). If further research replicates these early findings, then again, this information could be used in the development of training programmes. Regarding *ACTN3,* at present it appears that R allele carriers potentially exhibit greater increases in strength and power following high-load resistance training (Delmonico et al., 2007). As such, Kikuchi and Nakazato (2015) speculate that R allele carriers should prioritise high-load, low-repetition resistance training if improvements in muscle strength are required, and high intensity interval (HIT) training to specifically elicit improvements in VO_{2max}.

6. Conclusion

There is a clear, undoubted effect of genetic variation on both sporting performance and exercise adaptation. In this regard, one of the most well-studied genes is *ACTN3*, variation in which has been reliably shown to impact speed-power and strength phenotypes. However, emerging research indicates that this polymorphism may also affect other exercise associated variables, including training adaptation, post-exercise recovery, and exercise-associated injuries; this research is summarised in figure 6 below. This information is important, not just because it illustrates the wide-ranging impact SNPs can have, but also because it represents an opportunity to personalise, and therefore enhance, training guidelines. At present, there are no best-practice guidelines pertaining to the use of genetic information in both elite sport and the general public. However, sports teams have been using genetic information for over ten

years (Dennis 2005), and continue to do so. Consequently, the development of these guidelines represents an important step from lab to practice. Clearly, further research is required to fully develop these guidelines, and at present such information is speculative. Nevertheless, the use of genetic information represents an opportunity to enhance training prescription and outcomes in exercisers of all abilities.

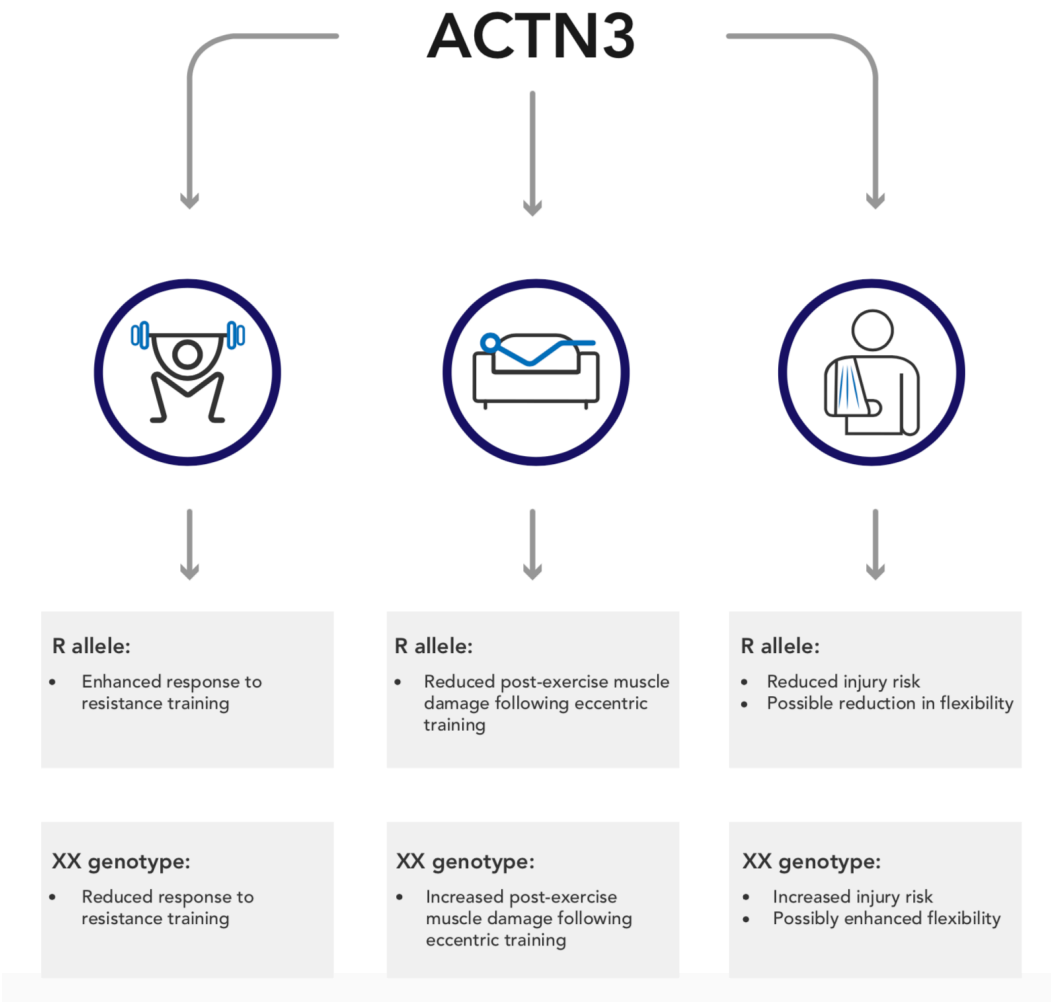

Figure 6 – A summary of the potential wider implications of *ACTN3* genotype on outcomes from exercise.

CHAPTER 7 - GENES, HAMSTRING INJURY, AND THE RESPONSE TO ECCENTRIC TRAINING

Chapter preface:

Hamstring injuries are prevalent within a diverse range of sports, and yet a plethora of research suggests that it should be reasonably easy to reduce their occurrence (Brukner 2015). In recent years, there has been an increased focus on the prevention of hamstring injuries within sport, with an emphasis on increasing the hamstring muscle's fascicle length and strength capabilities through eccentric loading. However, even with both this increased attention and evidence of the effectiveness of various interventions, hamstring injuries haven't declined appreciably within elite sport. One often cited reason for this lack of eccentric loading uptake and adherence is that of increased muscle soreness following eccentric loading, which, in the context of weekly competitions, often comes at a recovery cost. This chapter, which was published in *Medical Hypotheses* (Pickering & Kiely, 2018c), reviews the evidence of a genetic influence on the risk of hamstring injury, as well as both the adaptive and acute damage response to eccentric training bouts. Such information may better inform hamstring injury prevention techniques, which, given the current interest in this field, has the potential to be highly impactful.

1. Introduction

During the 2016/17 football season, there were 614 significant injuries recorded amongst the players of the twenty English Premier League clubs. These injuries resulted in a loss of over 20,000 training days, with the associated costs imposed in terms solely of injured player's wages exceeding £131 million. Over the course of this season, the most frequently injured site was the hamstring muscle group, representing 27% of all injuries suffered (Coates 2017). The ubiquity of hamstring strain injury (HSI) is not unique to soccer, and HSIs typically represent the most prevalent form of non-contact injury within competitive athletics (Edouard et al., 2016), American Football (Elliot et al., 2011), rugby union (Brooks et al., 2006), cricket (Orchard et al., 2002a), Australian Rules Football (Orchard et al., 2002b), and basketball (Meeuwisse et al., 2003). Alongside the substantial financial implications, HSIs also exert a large time-cost, with average recovery times ranging from 8 to 73 days depending on injury severity (Ekstrand et al., 2012). Furthermore, the unavailability of squad members due to injury diminishes team performance. As an illustration, in an eleven-season study of 24 European soccer clubs, lower injury prevalence was associated with a greater number of points gained per match, and a higher final league ranking (Hagglund et al., 2013). Perhaps most insidiously, prior HSI serves to increase the risk of further HSI (van Beijsterveldt et al., 2013), other injuries (Opar & Serpell, 2017), and future performance potential (Røksund et al., 2017). Consequently, avoiding, or at least reducing, HSI is a crucial consideration for many sports performance staff.

Although HSIs occur at varied locations within the muscle-tendon unit (MTU), the majority of injury mechanisms may be categorised within two broad classifications (Askling 2011). Firstly, and most commonly, HSIs occur during the late swing phase of high-speed running (Woods et al., 2004; Petersen

& Holmich, 2005), as the rapid and forceful deceleration of the lower leg severely increases hamstring tension (Petersen & Holmich, 2005, Chumanov et al., 2011). Such high-speed injuries tend to be located in the proximal portion of the MTU (Askling 2011). Conventionally, it is assumed that hamstring muscle fibres act eccentrically during this breaking action (Chumanov et al., 2011), as well as during the stance phase (Yu et al., 2008). This perspective, however, has recently been challenged, with an argument suggesting that the hamstring muscle fibres act isometrically during the swing phase (Van Hooren & Bosch, 2017). The other main provocative action occurs when the hamstring MTU is suddenly lengthened, for example during kicking, sliding, or sagittal splits activities (Askling 2011).

Given both the high frequency and associated costs of HSI, it is unsurprising that, in both academic and practical contexts much effort has been dedicated to answering two currently contentious, unresolved, and critical questions:

i) Is it possible to identify players most at risk of HSI? (Ruddy et al., 2017)

ii) What are the optimal physical training interventions to most productively enhance hamstring resilience? (Bourne et al., 2018)

In relation to screening for HSI risk, although some anatomical and historical features—such as age, (Ruddy et al., 2017), low levels of eccentric strength (Ruddy et al., 2017), muscle fascicle length (Timmins et al., 2016) and previous injury history (Gabbe et al., 2005)—have been associated with likelihood of HSI occurrence, developing tests with true predictive value has proven problematic (Bahr 2016). Similarly, given the assumed role of eccentric contractions in HSI aetiology, over a decade of empirical evidence supports the notion that the capacity to tolerate high forces during an increase in muscle length is an important aspect of HSI prevention (Bourne et al., 2018). These findings have led to the popularisation of exercises such as the Nordic hamstring exercise (NHE) (Al Attar et al., 2017) and Yo-Yo hamstring curl (Askling et al., 2003). Utilisation of these eccentric loading exercises has been shown to be effective in reducing the prevalence of HSI in athletic populations (Askling et al., 2003, Petersen et al., 2011, van der Horst, 2015; Al Attar et al., 2017), through the likely mechanisms of increasing eccentric strength and hamstring muscle fascicle length (Timmins et al., 2016; Bourne et al., 2018). Given these findings, eccentric hamstring exercises such as the NHE are increasingly prioritsed in elite sports programmes as an injury reduction tool (McCall et al., 2015), and as a potential means to enhance sprint performance (Ishoi et al., 2017). However, implementation of, and compliance with, these exercises is often problematic (Bahr et al., 2015), with concerns regarding increased muscle soreness, and a perceived lack of effectiveness, often cited by staff and players alike (McCall et al., 2015).

There is, however, an additional source of insight that may help both illuminate the answers to these questions, and, furthermore, may provide practitioners with meaningful guidance relating to the personalisation of injury prevention interventions. Previously, this thesis has argued that the utilisation of genetic information, alongside other more conventional measures, may aid in both explaining and predicting individualised training responses (Chapter 2; Pickering & Kiely, 2017a). Building on these previous arguments, this chapter widens the scope to investigate whether genetic information can contribute to the prediction of HSI, and in the personalisation of exercise interventions designed to reduce HSI incidence.

2. SNPs potentially involved in HSI

The influence of genetics on injury predisposition has been most well studied in relation to tendon and ligament injury, with SNPs in two genes, *COL1A1* and *COL5A1*, associated with an increased injury vulnerability (Posthumus et al., 2009a+b+c; Collins et al., 2009). There is, however, very little research examining the interaction of specific genotypes on skeletal muscle injury, and even less specifically looking at HSI.

Regarding muscle injuries in general, Pruna and colleagues (2013) examined the influence of 10 SNPs on the type and degree of injury in 73 professional elite male soccer players, playing for FC Barcelona, over three seasons. A total of 203 non-contact muscle injuries were recorded. Two SNPs, one each in *IGF2* and *CCL2*, were associated with muscle injury severity. *IGF2* acts to influence tissue repair (Keller et al., 1999), whereas *CCL2* is implicated in inflammation (Hubal et al., 2010). Consequently, variation in these genes may modify chronic load tolerance. Interestingly, when stratifying for ethnicity, an association between a SNP in *ELN* and injury severity emerged in Hispanics (Pruna et al., 2015), illustrating that, although in a low sample size (n=19), ethnicity is a potential modifier in the relationship between genetics and injury. *ELN* encodes for elastin, which is believed to modify tissue elasticity (Muiznieks et al., 2010). When these elite soccer players were followed for an additional two seasons, with further candidate SNPs analysed, additional tentative associations relating to injury prevalence for two SNPs in *HGF*, and one in *SOX15*, were established (Pruna et al., 2017). Regarding injury severity, the previously reported associations between *IGF2* and *CCL2* (Pruna et al., 2013) were replicated, and further associations uncovered for an additional four SNPs, one in *COL5A1* and three in *HGF*. *HGF* aids in the activation of muscle satellite cells (Pruna et al., 2017), and thus is likely implicated in skeletal muscle repair, as is *SOX15* (Pruna et al., 2017).

Similar to the work by Pruna and colleagues (2013, 2015, 2017), Massida et al. (2017) examined the effect of a single SNP in *ACTN3* on the frequency and severity of muscle injuries in 257 Italian male professional soccer players. *ACTN3* encodes for α-actinin-3, a protein that is an important component of the Z-disc (North et al., 1999). Individuals with the XX genotype cannot produce α-actinin-3, and so are believed to be predisposed to greater muscle damage following eccentric loading (Pimenta et al., 2012), potentially increasing injury risk. Furthermore, within this cohort, players with the XX genotype were significantly more likely to suffer an injury compared to R allele carriers (Odds Ratio = 2.66). These injuries were also significantly more likely to be of greater severity (OR = 2.13). In a smaller cohort of Italian footballers (n=173), a SNP in *MCT1* (rs1049434) was also significantly associated with muscle injury incidence (Massida et al., 2015). As *MCT1* is a lactate transporter, the proposed mechanism is that this SNP partially mediated muscle fatigue, a known injury risk-factor (Opar et al., 2012).

At present, only one paper has specifically examined the interaction between genotype and hamstring injury. Larruskain et al. (2017) recruited 107 elite male soccer players, recording hamstring injury prevalence from the start of the 2010-11 season until the end of 2014-15 season (5 seasons in

total). The players were genotyped for 37 SNPs previously associated with musculoskeletal injuries and/or exercise-induced muscle damage. Five SNPs were significantly associated with the risk of HSI in a multivariable model; *MMP3* (rs679620), *TNC* (rs2104772), *IL6* (rs1800795), *NOS3* (rs1799983), and *HIF1A* (rs1159465). Age (>24y) and previous hamstring injury were also risk factors for hamstring injury. However, whilst this model proved useful in explaining the prevalence of historical hamstring injury in the predictive stage of the study, it was found to be no better than chance at predicting future injury. As such, whilst it might be possible to retrospectively explain hamstring injuries through understanding genetic variation, it appears this information cannot be used to predict future injury, presumably due to the complex multifactorial nature of sporting injury (Bahr & Krosshaug, 2005).

In summary, a breadth of SNPs demonstrate tentative associations with muscle injury. However, few of these have been tested and/or associated with hamstring specific muscle injury in elite sportspeople. These SNPs come from genes influencing a variety of potential injury mechanisms, including muscle architecture (*ACTN3*) (Massida et al., 2017), muscle fatigue (*MCT1*) (Massida et al., 2015), inflammation (*IL6*) (Larruskain et al., 2017), and tissue repair and remodeling (*HGF* and *IFG1*) (Pruna et al., 2013; Pruna et al., 2017). Whilst these examples illustrate the complexly entangled influence of genetic factors on injury risk, as of yet utilisation of this information remains unable to predict future HSI (Larruskain et al., 2017).

3. A genetic influence on the response to mechanical loading

Adaptive responses to imposed exercise interventions vary extensively between individuals (Hubal et al., 2005; Erskine et al., 2010; Mann et al., 2014). This inter-individual diversity has been attributed to within-subject random variation (Atkinson & Batterham, 2015) and true between-subject neuro-biological variability (Mann et al., 2014). These true between-subject differences can be broadly characterised as genetically, environmentally, and epigenetically driven (Pickering & Kiely, 2017a), with heritable factors estimated to explain approximately 50% of the between-subject variance in strength (Silventoinen et al., 2008).

This phenomenon is most well explored in relation to concentric muscle contractions, the contraction mode most commonly used in general resistance training activities. Here, a number of genetic variants have been associated with modifying the training response. These include *ACTN3* (Delomonico et al., 2007; Pereira et al., 2013), *IGF1* (Hand et al., 2007), and *ACE* (Pescatello et al., 2006; Giaccaglia et al., 2008). However, there are also considerable inter-individual variations in response to both isometric and eccentric training. Heritable factors have been shown to account for between 14-83% of the variance in isometric strength (Peeters et al., 2009), with a value typically towards the higher end of this range often reported (Thomis et al., 1997; Tiainen et al., 2009). As with concentric contractions, numerous genetic variants have been associated with this phenotype, with *ACE* leading the way; in this case, the D allele appears to be associated with enhanced improvements following isometric loading (Folland et al., 2000).

3.1 Genetic insights into the response to eccentric loading

However, perhaps of greatest interest in terms of HSI prevention are eccentric training protocols. As discussed in the introduction, exercises designed to increase eccentric hamstring strength are popularly used within sport to reduce the prevalence of HSI (Askling et al., 2003). Such interventions have been shown to be effective (Petersen et al., 2011; Al Attar et al., 2017), with the proposed mechanism that they increase both the strength of the hamstring muscles (Mjolsnes et al., 2004), and also the muscle fascicle length (Potier et al., 2009; Reeves et al., 2009). As with other training modalities (Hubal et al., 2005; Erskine et al., 2010; Mann et al., 2014) the magnitude of improvement following eccentric training is likely to exhibit inter-individual variability (Baumert et al., 2016b), with differences in genotype partially explaining this variation (Moeckel-Cole et al., 2010).

3.1.1 Genetics and strength gains

Eccentric training appears to preferentially drive hypertrophy in type-II, and in particular type-IIx, muscle fibres (Douglas et al., 2017). Variation in muscle fibre type in general, and the magnitude of hypertrophy following training, is partially heritable (Simoneau & Bouchard, 1995; Timmons et al., 2010). One gene that exerts a relatively sizeable influence is *ACTN3*, where a common SNP results in a premature stop codon (X allele). Individuals with the XX genotype cannot produce the α-actinin-3 protein, which is expressed in type-II muscle fibres (North et al., 1999). As a result, these individuals typically present with smaller percentages of type-II fibres (Vincent et al., 2007), and appear to exhibit smaller improvements following resistance training (Delmonico et al., 2007). Subsequently, it seems feasible to suggest that the *ACTN3* XX genotype may attenuate gains in muscle strength following eccentric resistance exercise.

3.1.2 Genetics and muscle fascicle length

Alongside improvements in muscle strength, a further beneficial eccentric training adaptation is an increase in muscle fascicle length (Potier et al., 2008; Bourne et al., 2016). Again, inter-individual variation in this adaptation is likely to exist, with such variation partially genetically mediated. *TTN*, the gene encoding for the structural protein titin, may modify changes in muscle fascicle length. Here, a C>T transition at rs10497520 has been reported to modify muscle fascicle length in males (Stebbings et al., 2017), with CC homozygotes having longer vastus lateralis fascicles than CT heterozygotes. Whether this finding would be replicated in the hamstring muscle group, and whether it would affect changes in muscle fascicle length, remains to be elucidated.

3.1.3 Genetics and post-exercise recovery

Muscle damage: Alongside modifying the adaptive response to eccentric training, genetic variation may also affect recovery from such training. This was recently covered in a review by Baumert and colleagues (2016a). Here, genetic variation was determined to modify both the initial post-exercise damage phase and the subsequent inflammatory stage. In the initial damage phase, again *ACTN3* has been shown to play a role, with XX genotypes expected to exhibit greater muscle damage following exposure to eccentric loads (Vincent et al., 2010; Pimenta et al., 2012), although this finding remains equivocal (Venckunas et al., 2012). Here, the purported explanation is that the lack of α-actinin-3 in XX genotypes leads to weaker z-lines in type-II fibres, increasing their susceptibility to damage from eccentric contractions (Beggs et al., 1992; Friden & Lieber 2001; Seto et al., 2011a). Other SNPs that appear to affect muscle damage during eccentric contractions include two in *MLCK* (rs2700352 and rs28497577) (Clarkson et al., 2005a), and one in *CK-MM* (rs1803285), although this SNP has thus far yielded conflicting results (Heled et al., 2007; Yamin et al., 2010; Deuster et al., 2013).

Inflammation: Genetic variation can also predispose individuals to increased inflammation following eccentric exercise (Baumert et al., 2016a). Many of these SNPs are from the interleukin family, with a polymorphism in *IL6* (rs1800795) perhaps the most prominent. Here, the C allele is associated with a greater increase in creatine kinase (CK) activity following maximal eccentric contractions (Yamin et al., 2008). Genes encoding for other pro-inflammatory cytokines, such as tumor necrosis factor (TNF), also modify the post-training inflammatory response following exercise (Lakka et al., 2006; Yamin et al., 2008), and appear likely to influence recovery following eccentric.

Taken together, it is clear that genetic variation influences multiple dimensions of eccentric exercise recovery. This can be in terms of muscular damage, for example though the mediation of *ACTN3* (Pimenta et al. 2003), or modulation of the inflammatory response, exemplified by *IL-6* (Yamin et al., 2008).

Muscle soreness: A feature of un-habituated eccentric exercise is that it typically results in muscle soreness (Lee et al., 2002). This is one of the often-cited reasons why elite athletes, despite the demonstrated value of eccentric hamstring exercise, have historically been slow to engage in such training (McCall et al., 2015). Accordingly, information relating to the likelihood of suffering from post-eccentric exercise discomfort could be useful. If the magnitude of soreness following eccentric loading can be predicted—even partially—then training interventions can be adjusted to promote engagement and adherence accordingly. In the case of acute muscle damage, for example, this could inform the individual calibration of training volumes and/or intensities. Here, knowledge of *ACTN3* genotype may be helpful, with XX homozygotes expected to experience greater levels of soreness. Of further relevance, a second SNP in *TTN*, rs11693372, may affect post-eccentric muscle soreness, with the CC genotype protective against subjective soreness (Moeckel-Cole et al., 2010).

This information may also be useful in-season, with those players predicted to experience increased soreness being guided to undertake eccentric loading exercises further away from a competition

or match-play. The same is true for the inflammatory response, which modulates recovery time, and influences soreness (Miles et al., 2008). In this case, personalised nutrition guidelines could be formulated based on genotype. Here, individuals with a genetic predisposition to an increased inflammatory response may increase intake of flavonoids, omega-3 fatty acids, and other nutrients associated with a reduction in inflammatory biomarkers following eccentric exercise (Phillips et al., 2003; DiLorenzo et al., 2014; Kim & Lee, 2014). Such genotype-based nutritional interventions have yet to be tested in sports people, but a number of SNPs – including *ACTN3, CM-MM, IL6,* and *TNF* – have been utilised as part of a Total Genotype Score (TGS) to explain individual variations in the level of muscle damage (Del Coso et al., 2017a & b) following endurance activity.

4. Conclusion - Using this information

Whilst certain genetic variants may increase the predisposition to HSI (Pruna et al., 2013; Larruskain et al., 2017), as of yet it does not appear possible to use genetic information to predict HSI occurrence (Larruskain et al., 2017). This lack of predictability reflects the complex, multifactorial nature of sporting injuries (Bahr & Krosshaug, 2005; Bahr 2016). At present, it therefore appears difficult to make specific recommendations based on an athlete's genetic predisposition to HSI, because genetic variation appears to explain very little of the between-athlete variance in HSI prevalence. That said, whilst injuries cannot be accurately predicted—such that all "at-risk" athletes get injured, and no "low-risk" athletes do—genetic information could be used alongside other, more traditional methods such as acute:chronic workload (Hulin et al., 2016a) and eccentric strength testing (Sugiura et al., 2008) to develop a clearer picture of individual risk, perhaps guiding the customisation of hamstring robustness-enabling interventions.

As discussed, athlete genotype potentially modifies training adaptations to eccentric loading (Moeckel-Cole et al., 2010), as well as altering the acute inflammatory (Yamin et al., 2008) and muscle-damage (Del Coso et al., 2017a) response to such exercises. Accordingly, there remains the possibility that genetic information, although inadequate as a predictive tool for HSI, can instead enable a more informed application of preventative exercises. In this scenario, genetic information could be used to better inform loading schemes (Kikuchi & Nakazato, 2015; Jones et al., 2016) and recovery strategies. This could be especially important during the competitive season, when avoiding excessive post-exercise soreness prior to key competitions and matches is crucial. In this case, genetic information could be used alongside more conventional measures in order to optimally position the eccentric loading bout within the training week for that athlete. Similarly, utilising genetic information may aid in the process of introducing this training modality to eccentric-naïve individuals, with coaches using information relating to post-exercise soreness to modify the load and intensity accordingly.

Such a hypothesis remains largely untested, representing an avenue for future research. This is of increased importance given the lack of intervention-based studies in the field of sports genetics, and represents an ideal opportunity to move from observational research to that which directly impacts practice (Buchheit, 2017), particularly as both athletes and coaches appear amenable to the utilisation of

genetic information (Varley et al., 2018a). In moving this field forward, future research should therefore aim to elucidate:

1. The extent to which specific genetic variants modify the HSI risk.
2. Whether knowledge of this information can be predictive in terms of HSI probability.
3. Whether modification of training variables based on genotype leads to better outcomes following eccentric hamstring exercises in terms of injury resilience and athletic performance.

Given the prevalence of HSI within elite sport, such insights have the potential to inform and enhance hamstring performance and robustness training process. Whilst not predictive of HSI injury in and of themselves, genetic variants do provide insights into the likely predispositions certain athletes may have to such to injury, and subsequently provide an additional layer of relevant information that can be combined with more conventional assessments to guide the customisation of hamstring-specific exercise prescription and monitoring strategies. Despite the recent surge in HSI research, it remains clear that hamstring injuries still cannot be prevented (Ekstrand et al., 2016). Solving such a complex, multi-factorial phenomenon will likely demand the integration of insights and information from multiple domains. In pursuing this objective, there is the potential that an appreciation of the underlying genetic mechanisms influencing HSI risk and training responsiveness will provide a useful—albeit partial—insight that can positively contribute to more perceptive management of hamstring health.

CHAPTER 8 – CAN GENETIC TESTING IDENTIFY "TALENT" (WHATEVER THAT MIGHT BE)?

Chapter preface:

The use of genetic information to identify future talented performers represents a potential "holy grail" within the talent identification sphere. Indeed, some nations are already utilising genetic information in this way, even though the general scientific consensus is that genetic testing for talent holds no predictive ability (Webborn et al., 2015), and is ethically troubling (Camporesi & McNamme, 2016; Vlahovich et al., 2017a). This chapter explores the use of genetic information as a talent identification tool, and is split into two parts. Part One asks whether genetic information could ever be used for talent identification, providing an overview of the challenges in creating an evidence based genetic testing programme for talent identification. Part Two explores talent identification from a different perspective, asking whether the ability to positively, and substantially, adapt to exercise can be considered a talent—and if so, is it possible to test for it? Part One was previously published in *Genes* (Pickering et al., 2019b), and Part Two was previously published in *Sports Medicine Open* (Pickering & Kiely, 2017c).

PART ONE – COULD GENETIC INFORMATION EVER BE USED FOR TALENT IDENTIFICATION?

1. Introduction

Elite athlete status is a partially heritable trait, with approximately 66% of the variance between elite and non-elite athletes explained by heritable factors (De Moor et al., 2007). Furthermore, recent advances in genetic technology have allowed for greater exploration of the genetic underpinnings of elite performance. This has led to the identification of a number of single nucleotide polymorphisms (SNPs) and other genetic variants with the potential to affect performance, both directly and indirectly. For example, a SNP in *ACTN3*, R577X (rs1815739) has been shown to impact attainment of elite speed-power athlete status (Ma et al., 2013). Here, a common C-to-T base substitution results in the transformation of an arginine base (R) to a premature stop codon (X). X allele homozygotes are deficient in the protein encoded for by *ACTN3*, α-actinin-3, which is expressed exclusively in fast twitch muscle fibres (North et al., 1999). As a result, these XX genotypes tend to have lower proportions of fast-twitch muscle fibres (Vincent et al., 2007), and, given that fast-twitch muscle fibres are an important component of speed-power performance, tend to be underrepresented in elite speed-power cohorts (Yang et al., 2003). The first study demonstrating this was conducted by Yang and colleagues (2003), who reported that the X allele was significantly underrepresented in a cohort of elite male and female sprint athletes when compared to both non-athletic controls and elite endurance athletes. Whilst in Caucasian populations the frequency of the XX genotype is ~20% (Yang et al., 2003), in Yang and colleague's (2003) cohort of Caucasian power Olympians, it was entirely absent. The finding of significantly lower X allele frequencies and XX genotypes in elite speed-power athletes has been well replicated

(Druzhevskaya et al., 2008; Roth et al., 2008; Eynon et al., 2009c; Ahmetov et al., 2011; Cieszczyk et al., 2011), although equivocal findings have also been reported (Gineviciene et al., 2001; Sessa et al., 2011; Scott et al., 2010). Consequently, despite explaining "only" around 3% of the variance in speed-power phenotype (Moran et al., 2007), *ACTN3* has subsequently been labelled a "gene for speed" (MacArthur & North, 2004; Berman & North 2010; Chan 2008). *ACTN3,* however, is not the only gene associated with elite athlete status, with a recent review reporting that at least 155 genetic markers have been linked to elite athlete status (Ahmetov et al., 2016).

Whilst many of the currently established SNPs associated with elite athlete status are linked to physiological traits such as speed, aerobic endurance, and strength, there is the potential that other SNPs may exert a less direct—but no less crucial—impact on the attainment of elite performance. For example, both height (Silventoinen et al., 2003) and Body Mass Index (BMI) are highly heritable (Allison et al., 1996; Elks et al., 2012); and both likely contribute to the attainment of elite athlete status on a sport-by-sport basis. Furthermore, psychological traits are also genetically influenced, with a number of SNPs associated with anxiety (Stein et al., 2005; Chen et al., 2006; Clasen et al., 2011). As such, the genetic influence on performance is broad, multi-factorial, and pervasive.

Given the wide-ranging and potentially powerful influence of genetic variants on both the attainment of elite athlete status and the development and possession of the individual physiological, psychological and biomechanical traits that underpin elite performance, there is considerable interest in collecting genetic information in order to identify athletes with the potential to achieve elite status. Indeed, a number of direct-to-consumer (DTC) companies offer such genetic testing (Collier 2012; Wagner & Royal, 2012; Roth et al., 2012; Webborn et al., 2015), and contemporary reports detail the use of genetic testing within the talent identification process in a number of countries (https://www.newsweek.com/china-begin-using-genetic-testing-select-olympic-athletes-1099058). However, at present the general consensus amongst researchers is that such tests have no role to play in talent identification (Webborn et al., 2015; Vlahovich et al., 2017a), and are also ethically troubling (Miah & Rich, 2006; Camporesi & McNamee, 2016). This chapter section discusses why current genetic tests cannot predict future sporting success, and explores what advancements would be required to enable the utilisation of genetic information to more accurately identify future talented performers.

2. Why can't genetic information currently be used for talent ID?

As previously mentioned, over 155 genetic markers have been linked with elite athlete status (Ahmetov et al., 2016). These markers are typically—but not always—divergent, such that they predispose towards an increased chance of success in either power-strength or endurance sports/events, but not both. These divergent effects demonstrate that there isn't a singular genetic profile that confers sporting success, but that the required genetic profiles are likely specific to individual sports and events. Whilst some of these markers, such as *ACTN3* (Yang et al., 2003; Druzhevskaya et al., 2008), *ACE* (Gayagay et al., 1998; Jones et al., 2002), and *PPARGC1A* (Lucia et al., 2005; Maciejewska et al., 2012) are well established and well replicated, others, such as *TFAM* (rs1937), have yet to be satisfactorily

replicated (Ahmetov et al., 2010). Currently, only a few of the genetic markers that likely associate with elite athlete status have been identified, making predictions of future sporting prowess based on such information both difficult and incomplete.

Another issue is that, at present, the currently available markers appear to offer poor specificity and sensitivity as talent identification tools. Returning to *ACTN3*, whilst it is clear that the R allele is associated with elite athlete status in speed-power events (Ma et al., 2013), with the XX genotype significantly less common in such cohorts (Yang et al., 2003; Papadimitriou et al., 2008; Druzhevskaya et al., 2008), it remains unclear how discriminatory this information might be. In Caucasians, for example, ~80% of individuals possess an R allele (Yang et al., 2003). In some black African populations, this percentage can be as high as 99% (Yang et al., 2007). In a study of elite US and Jamaican sprinters—the populations providing the fastest eight 100m runners of all time—there was no difference in *ACTN3* genotype frequencies between these athletes and non-athlete controls, with 97% of non-athletes possessing at least one R allele (Scott et al., 2010). So, whilst the R allele may be required for elite sprint performance, given that the vast majority of the world's population possess it, this knowledge is not particularly useful. Furthermore, there are exceptions to the belief that an R allele is required for elite speed-power performance. In their study of elite sprinters, Papadimitriou and colleagues (2016) reported that one male and one female 100m sprinter, both of whom achieved the Olympic qualifying standard, did not possess an R allele. Additionally, Lucia and colleagues (2007) reported the case of a long jumper with a personal best of 8.26cm, just 5cm off the gold medal winning jump at the 2012 Olympic Games, who possessed the XX genotype. Such findings demonstrate that the lack of an *ACTN3* R allele does not preclude elite status in speed-power events. Additionally, many such performance enhancing polymorphisms may still have a low prevalence in elite athlete populations. For example, a SNP in *NRF2* (rs7181866) has been associated with elite athlete status, with a significantly higher proportion of the AG genotype compared to the AA genotype found in elite endurance athletes when compared to controls (Eynon et al., 2009b). However, only 12-14% of these elite athletes possessed the "ideal" AG genotype, illustrating that the vast majority of elite athletes were not in possession of this specific performance enhancing polymorphism, limiting its use as a discriminating screen.

Such findings demonstrate the problems of a single gene approach to talent, and, indeed, no serious researcher or practitioner today would consider such an approach viable. In light of these findings, researchers have turned to Total Genotype Scores (TGS), whereby a number of elite athlete-associated SNPs are combined into a single polygenic score. Ruiz and colleagues (2009; 2010) utilised such an approach involving elite endurance and power athletes. For their endurance study, they combined seven polymorphisms into a total score, finding that the mean score was higher in the athlete group compared to the control group (Ruiz et al., 2009). This finding was replicated using a TGS comprised of six SNPs for power athlete status, with elite power athletes having a higher score than both endurance athletes and non-athletic controls (Ruiz et al., 2010). Possession of the "perfect" polygenic profile (i.e. the elite athlete genotype of all SNPs) was rare, occurring in only 9.4% of the power athletes, and no endurance athletes. In addition, there was considerable overlap between groups, such that a number of controls had better TGS than elite power athletes, as did a number of elite endurance athletes (Ruiz et al., 2010). Furthermore, whilst a TGS may help in discriminating between athlete and non-athlete, Santiago and

colleagues (2010) demonstrated that, in a group of rowers, it did not distinguish between different levels of performance (i.e. World vs National medallists). Earlier work (Williams & Folland, 2008; Hughes et al., 2011) demonstrates that there is considerable similarity in polygenic scores within humans—athlete and non-athletes alike—when a low number of markers (22-23) are used, such that, again, this approach would likely have limited real-world specificity and sensitivity. In order to improve the insights provided, a far greater number of performance-enhancing polymorphisms are likely required.

As a summary of the above discussion, it's clear the provision of elite athlete status is a highly complex, polygenic trait, and that, at present, very few of the genetic variations that contribute to this trait have been identified. As a result, it appears a fundamental requirement that, if genetic testing is to be utilised for talent identification purposes, a far greater number of polymorphisms associated with elite athlete status need to be uncovered, and then combined into a TGS model.

3. What further knowledge is required to *potentially* use genetic information for talent identification?

3.1. Genome-Wide Association Studies

The evolution of Genome Wide Association Study (GWAS) methodology potentially offers an opportunity to expand the number of genetic variants currently associated with elite athlete status. Whilst the majority of the SNPs currently associated with elite athlete status were elucidated via gene-association studies or candidate gene analysis—where a SNP is hypothesised to have an effect, and that hypothesis is then tested—GWAS are hypothesis-free. In a GWAS, a large number of SNPs (e.g. ~700,000) are analysed for association with a trait. Because there is no hypothesis to be tested, it provides a robust method to detect novel associations. However, due to the very low p-values required to reach genome-wide significance ($p<5x10^{-8}$), and the (often) very low effect sizes of any individual SNP, GWAS analyses often require very large subject numbers. This is problematic when it comes to research on elite athletes, who are, by definition, rare. Such a problem was encountered in a GWAS carried out in multiple cohorts of elite endurance athletes by Rankinen and colleagues (2016). Here, the authors utilised a cohort of elite endurance runners (n=315) and controls, known as GENATHLETE, along with a cohort of elite Japanese runners (n=60) and controls, for the discovery phase of the GWAS. Following this discovery phase, in which no SNP met genome-wide significance, forty-two suggestive SNPs were taken through to a replication phase involving endurance athletes and controls from seven other countries. Again, no genome-wide significant SNPs were found. As such, the authors summarised that there appeared to be no common SNP associated with elite endurance athlete status across this cohort, although they acknowledged their low sample size as a limiting factor. Such a limitation is difficult to overcome, and represents a significant roadblock in the search for genetic variants associated with elite performance. A further potential roadblock is that there may be different associations between SNPs and elite performance across ethnicities, such that a SNP may be performance enhancing in Caucasians, but not East Asians, for example, thereby requiring the development of ethnicity-specific SNP panels for the purpose of talent identification.

3.2. Rare variants

A further avenue for exploration is that of rare genetic variants that may predispose to elite performance. Generally, research focuses on fairly common polymorphisms, present in >1% of the population (Moran & Pitsiladis, 2017). However, there are a few genetic variants identified which are very rare, and yet have the potential to impact performance. One such variant occurs at rs121917830 within the EPO receptor gene. This variant is linked to a disease called erythrocystosis-1, where sufferers have increased erythropoietin expression, and hence greater oxygen carrying capacity (Moran & Pitsiladis, 2017). This is potentially advantageous for endurance sport, and at least one elite athlete, Finnish cross-country skier Eero Mantyranta, possessed this variant (Juvonen et al., 1991). An additional example is that of variation in *LMNA*, a gene related to muscular dystrophy, that was found in Canadian sprint hurdler Priscilla Lopes-Schliep (Waggot et al., 2016). Finally, a rare variation in the myostatin gene (*MSTN*) has been reported, where carriers are described as "extraordinarily muscular". (Schuelke et al., 2004). Such a variant would potentially be advantageous in sports/events demanding high levels of strength or increased muscle mass. One issue with the exploration of rare performance enhancing variants is that, given their very low frequency, they can be hard to identify, and, in many cases, are only reported a handful of times in the research literature. Furthermore, they may also predispose to disease states; a factor raising complex moral and ethical questions. However, despite these potential issues, research continues towards their identification (Waggot et al., 2016), not least because identification of healthy individuals with disease-causing variants could provide information relating to the underpinning mechanisms of these diseases, and potentially inform remedial and resilience-building strategies (Chen et al., 2016).

3.3. Signal or noise?

As the number of variants associated with elite athlete status grows, it will be important to distinguish which of these are potentially causal, and those which are "noise" (Pickering & Kiely 2017b). For example, recently it was reported that the C allele of rs12722, a SNP within *COL5A1*, was more frequent in a cohort of elite rugby players compared to controls (Heffernan et al., 2017). This SNP has previously been associated with soft tissue injuries, with the T allele increasing the prevalence of such injuries (Mokone et al., 2006; Posthumus et al., 2009c; September et al., 2009). A potential explanation for this increased frequency in elite players is that the avoidance of injury is important for the attainment of elite status, and therefore a lower predisposition to injuries is advantageous (Heffernan et al., 2017). It is important to consider whether such a SNP should form part of a genetic test for talent, because injury risk itself is highly modifiable through environmental changes, such as increased exposure to eccentric loading (Fyfe & Stanish, 1992; LaStayo et al., 2003). As a result, possession of the protective allele for this SNP may only confer an advantage towards elite athlete status if carriers suffer fewer injuries, which can be directly modified, as opposed to a direct effect of this SNP on performance.

3.4. A predictive threshold?

An area of potential previous confusion is the number of performance-enhancing polymorphisms an individual may require before they are capable, from a genetic standpoint, of elite performance. Both Williams and Folland (2008) and Hughes and colleagues (2011) report that, in panels containing 23 (endurance) and 22 (strength-power) polymorphisms, the chances of one athlete possessing all performance-enhancing alleles was vanishingly small. As such, it seems unlikely that a single athlete possesses the "perfect" genetic profile, although such a perfect profile is arguably unnecessary. Instead, athletes will likely possess a given number of performance-enhancing polymorphisms. Crucially, the polymorphisms possessed will differ between athletes, such that there might be only minimal overlap between individuals. In this way, once a large number of SNPs responsible for driving elite athlete status are uncovered—should such discovery ever occur—there will be the potential for the development of a threshold number, whereby possession of polymorphisms above this number would be associated with elite performance. Accordingly, there will not necessarily be commonality in terms of the genetic variants present, although some crossover will certainly occur; instead, the main driving factor will be the total frequency of performance driving variants. These SNPs will also likely differ between ethnicities, and so ethnicity-specific thresholds and genetic panels will be required. The utilisation of a large number of SNPs reduces the reliance on individual SNPs that occur either at high frequencies across populations (such as the *ACTN3* R allele), or those that, whilst linked to elite athlete status, are still present at relatively low frequencies in elite cohorts (for example the *NRF2* G allele).

3.5. Epigenetic modifications

Finally, there is the potential that epigenetic modifications—changes in genetic expression that are not due to changes in the underlying genetic code—may affect the attainment of elite athlete status (Ehlert et al., 2013). These epigenetic changes are typically comprised of DNA methylation, histone modifications, and non-coding RNAs, particularly microRNAs (miRNAs) (Ehlert et al., 2013; Voisin et al., 2015). For example, miRNAs have been shown to modify the magnitude of exercise adaptation (Davidsen et al., 2010; Kirby et al., 2013), which is an important component of the journey towards elite athlete status. As of yet, whilst it appears that epigenetic changes may modify exercise adaptation (Denham et al., 2013; Ehlert et al., 2013, Voisin et al., 2015), it is not clear specifically what modifying effect they may have on the attainment of elite status. Additionally, such modifications have the potential to be passed down through generations (Richards 2006; Rissman & Adil, 2014), and thus may form part of the heritable aspect of elite athlete status. As a result, the ability to test for epigenetic changes, which are often tissue specific, could assist in the identification of talented athletes.

3.6. Lessons from disease prediction

One area where the use of genetic information to make informed predictions of a future event has been well explored is that of disease risk. Whilst some diseases, such as cystic fibrosis, are monogenic diseases, most are complex and polygenic in nature (Chatterjee et al., 2016). Similar to elite athlete status, whilst many diseases have been shown to have a large genetic component, the disease-

causing variants identified to date often explain little of the variance between individuals; this issue is referred to as the missing heritability problem (Manolio et al., 2009). One suggested method to overcome this problem of missing heritability is to lower the threshold for discovery of SNPs affecting the trait of interest. Due to the high number of comparisons carried out in a GWAS, statistical significance for discovery of new variants is typically set at $p<5x10^{-8}$. However, the lowering of this threshold has been shown to lead to explanation of a greater proportion of heritability (Yang et al., 2010; Shi et al., 2016; Boyle 2017). Recently, Khera and colleagues (2018) utilised a TGS comprised of 6,630,150 polymorphisms to create a risk score for coronary artery disease that had an area under the curve of 0.81, suggesting a strong predictive ability; many of these polymorphisms had miniscule effect sizes and weak significance, and yet combined to produce a powerful predictive tool. Such a method clearly holds promise for traits that have a large—but poorly elucidated—genetic component (Dudbridge 2013), such as elite athlete status. Indeed, returning to the recent GWAS on elite endurance status (Rankinen et al., 2016), whilst no SNP was discovered at the genome-wide significance level ($p<5x10^{-8}$), a number of SNPs had suggestive significance, and may hold predictive ability as part of a TGS. As a result, it appears likely that, in order to successfully predict future elite athlete status, models involving genetic variants with low effect sizes are likely required. However, the common issue of sample size returns; for discovery of relatively common genetic variants with small effect sizes, sample sizes in excess of 10,000 individuals are likely required (Mattsson et al., 2016)—a number likely greater than that of all truly elite athletes on the planet.

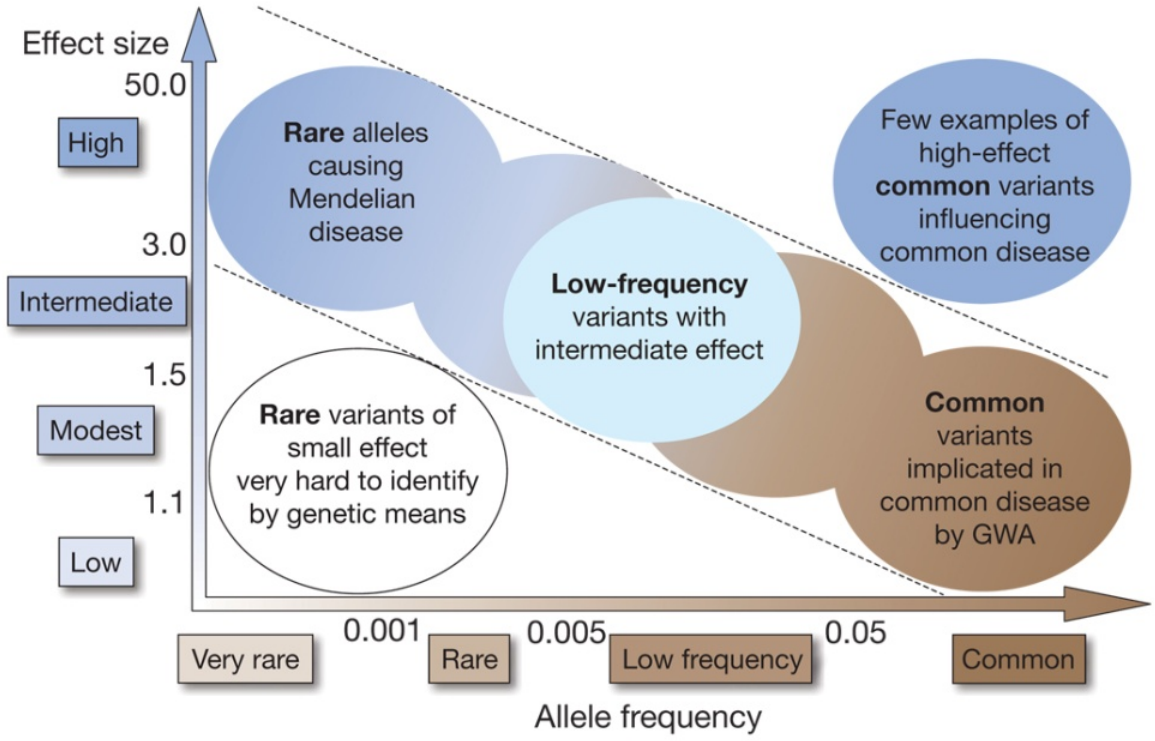

Figure 7 - A demonstration of the identification of risk variants for disease. The main focus on interest is within the dotted line. For the prediction of elite athlete status, rare alleles are likely not useful, given their lack of prevalence. Instead, there needs to be a focus on common genetic variants with low effect sizes – which require large sample sizes to be identified. (Figure taken from Manilo et al., 2009 and reproduced with permission).

4. Is genetic testing for talent ethical?

There are serious and well-placed concerns about the use of genetic information for talent identification within sport (Webborn et al., 2015; Camporesi & McNamee, 2016; Williams et al., 2016; Vlahovich et al, 2017a+b). It is generally considered that, within sporting contexts, genetic testing should not be carried out on under-18s (Webborn et al., 2015; Vlahovich et al., 2017a+b). If genetic testing for talent does become evidence based, then there will be a requirement for the development of guidelines on its use, in part to protect vulnerable young children. For example, should sports clubs be able to demand players undergo genetic testing as part of their talent identification programmes? Who can give informed consent, and are young children even developed enough to give such consent? Who should have access to the genetic data? Will it be used in a discriminatory way? What if a player refuses to undergo a genetic test? What happens if genetic testing uncovers a potential disease-causing variant? The latter point is potentially an important issue, as whilst it could lead to health-promoting medical interventions, it could also lead to unnecessary medical explorations, as well as increased worry, both on the part of the athlete tested and their relatives, who may also carry the disease-causing variant. Additionally, it would in theory be possible to subject embryos to a genetic test, and, if the desired mix of sporting genes are not present, abort it. Such outcomes are highly unpalatable, and likely represent an extreme example, but demonstrate the potential mis-use of such information.

Furthermore, if and when genetic testing is used to predict future elite athlete status, there will be many false positives and false negatives; i.e., many individuals will be mis-attributed to future elite or non-elite status (Breitbach et al., 2014). Whilst such error rates may be acceptable at a population level, they are obviously troubling at an individual one; who has the right to tell a young athlete that they don't have the genetic potential to succeed? Perhaps more importantly, what effect would receiving this information have on that individual's future exercise behavior, which, given the wide-ranging health benefits of exercise (Pareja-Galeano et al., 2015) is an important consideration for lifelong health.

5. What *could* genetic testing potentially be used for?

The second part of this chapter argues that, instead of using genetic information for talent identification in the traditional sense, it could be utilised to identify those athletes with the greatest capacity to improve with training (see also Pickering & Kiely, 2017c). Furthermore, this information could also be used to match individuals to the type of training to which they are most suited, and from which they will elicit the greatest adaptations (Jones et al., 2016; Pickering & Kiely 2017c). Additionally, genetic information could be utilised to identify those athletes with an increased risk of injury, allowing the provision of pre-emptive strategies to reduce that risk. For example, Varley and colleagues (2018b) identified a number of polymorphisms associated with an increased stress fracture risk in a cohort of elite athletes; in this case, the high-risk athletes could undergo additional bone mineral density monitoring, along with targeted interventions, such as vitamin D and calcium supplementation (Lappe et al., 2008).

Furthermore, whilst traditionally research has focused on the physiological drivers of elite athlete status, there is the potential to explore the genetic underpinnings of psychological factors, such as anxiety, stress resilience, and skill acquisition. Whilst this has not yet been explored in detail, a SNP within *COMT,* rs4680, has been linked with competition performance in swimmers (Abe et al., 2018) and personality traits in ultra-endurance athletes (van Breda et al., 2015). This gene encodes for catechol-o-methyltransferase, which plays a role in the regulation of dopamine within the prefrontal cortex (Stein et al., 2006); variation in this SNP affects dopamine levels, which can alter information processing and memory (Stein et al., 2006). Emerging research has also implicated a number of polymorphisms in altering the skill acquisition process (Jacob et al., 2018). Finally, a number of genetic variants have been linked to an increased susceptibility to concussion injuries (McFie et al., 2018; Abrahams et al., 2018). As a result, whilst this information could be used to bias against those with the perceived "unfavorable" genotypes, it could also be used to personalise the training process, identifying those athletes who need greater attention in these areas, and assisting in injury management and monitoring. Furthermore, with regards to both injury and concussion, the information could be used to better inform preventative methods, along with increasing the personalisation of recovery and return-to-play protocols, particularly given the evidence that genetic information may enhance adherence to interventions (Nielsen & El-Sohemy, 2014).

6. Conclusion

Whilst there is a strong and well-replicated modifying effect of genetic variation on the attainment of elite athlete status, based on the available evidence, it is clear that the current use of genetic tests for the prediction of future elite athlete status is ineffectual, a finding that echoes recent consensus statements (Webborn et al., 2015; Vlahovich et al., 2017a). In order to be able to use genetic information within the talent identification process, a far greater number of performance-enhancing polymorphisms need to be both discovered and replicated. The combination of these performance enhancing polymorphisms into a TGS, especially if the evidence threshold is lowered, appears to offer a solution to the limited predictive capabilities of small numbers of genetic variants. As the evidence base grows, it should be possible to determine a TGS threshold, above which an individual's chance of achieving elite athlete status in a given sport or event is higher. However, and this is a crucial point, there will be individuals with a score below this threshold who go on to achieve elite athlete status, and those with scores above the threshold who will not be elite athletes. Because elite athlete status is a manifestation of a number of variables, not just genotype, it seems unlikely that it will ever be possible to use genetic information to identify a future elite athlete with certainty. At best, genetic information may represent a potentially useful adjunct to existing talent identification procedures, enhancing the process, particularly as genetic information is not subject to some of the issues that commonly plague traditional talent identification processes, such as maturation and training age. Additionally, and as argued in the next section, genetic information may be used in the future to identify those with the greatest potential to show favorable adaptations to training (Pickering & Kiely, 2017c), as well as determine the optimal training type to elicit such adaptations (Jones et al., 2016). Furthermore, there is the potential to utilise such information to reduce injury occurrence (Heffernan et al., 2015). Again, and this point must be clear; such

information should not be used as a standalone, but as an adjunct, to current talent identification processes, thereby allowing the training process to become more personalised, and enabling athletes to get ever closer to their maximum potential.

PART TWO - CAN THE ABILITY TO ADAPT TO EXERCISE BE CONSIDERED A TALENT—AND IF SO, CAN IT BE TESTED FOR?

1. Introduction

The accurate identification of youth sporting talent has, in recent decades, emerged as a hugely important and yet controversial topic (Vaeyens et al., 2008; Issurin et al., 2017). Interest in Talent Identification (TI) is illustrated by a growing academic literature (Vaeyens et al., 2008; Collins et al., 2016; Issurin et al., 2017), along with a number of best-selling popular-science books on the topic (Colvin 2008; Coyle 2010; Syed 2010; Ericsson & Pool, 2016). Traditionally, sporting TI programmes have, through a mix of subjective and objective tests, sought to identify young athletes with "talent", using this identification as a prediction of adult performance. However, despite the massive allocation of resources into the identification and development of young talent, it remains unclear whether or not early TI processes are either empirically justified or practically effective.

One fundamental limiting factor is that physical performance tests employed to discern between those who have the talent to excel in the future, and those who do not, actually only provide a snapshot of current abilities. The subsequent logical leap is the presumption that those who perform well at that given time are most likely to be successful as adults. Yet, due to the inherently non-linear complex nature of biological maturation, these performance snapshots offer inherently poor predictive value.

The reasons why countless high performing youth and junior athletes do not maintain their relative early high performance standards are obviously complex, varied and multifactorial (Abbott & Collins, 2002; Abbott et al., 2005). This illustrates the gross inaccuracies associated with current approaches to predicting future senior potential based on youthful performance. Similarly, where TI processes have been empirically evaluated, these inefficiencies remain, with fewer than 2% of athletes identified as having the potential to be elite within a school sports programme winning senior international medals (Vaeyens et al., 2009).

Despite these inefficiencies, however, clubs and organisations invest large sums on TI and development initiatives in the hope of unearthing future talent. Manchester City's Academy programme, for example, reportedly costs £12m per year to run (Ashton 2017). Yet such large investment is perceived as both economically feasible and justified by the occasional unearthing of exceptional talent; over 15 Manchester City Academy graduates have been capped at senior international level, and one, Shaun-Wright Phillips, was sold by the club for £21m.

A clear limitation of the TI process is that, during maturation, current performance is not directly indicative of future potential. In fact, no standard physical assessment provides insight into how an individual is likely to respond to future training. This chapter section explores the possibility that the utilisation of genetic markers associated with the capacity to favorably respond to imposed training stress may provide valuable, and currently missing, insights relating to future trainability, rather than current ability; thus providing clues as to whether the athlete has the innate "talent" to respond to training.

2. The hereditary aspect of talent

A standardised, widely accepted definition of talent is hard to find. A review of the complexities surrounding an adequate definition of talent is beyond the scope of this chapter; however, Issurin recently utilised a broad definition of talent as "*a special ability that allows someone to reach excellence in some activity in a given domain*" (Issurin 2017). In conceptualising this definition, Issurin leaned heavily on Howe and colleagues (1998), who proposed that talent has five properties; it is partially innate; its full effect may not be evident at an early stage; it has early indications that provide a basis for predicting who might excel; only a few possess it; and it is domain specific.

Implicit within any definition of talent is the assumption that it is at least partially genetically determined. This is most obvious when considering the physiological underpinnings of elite performance, all of which are, to some degree, genetically influenced. Approximately 50% of variation in baseline maximal oxygen uptake (VO_{2max}) is heritable (Bouchard et al., 2000), as is 45-99.5% of muscle fibre type (Komi et al., 1977; Simoneau & Bouchard, 1995). Furthermore, variation in muscle strength is estimated to be ~52% heritable (Zempo et al., 2017). Anthropometric qualities, often used as TI indicators, are also genetically mediated, with variation in height approximately 80% heritable (Silventoinen et al., 2003). So too are non-physical traits associated with elite performance; for example, stress resilience has a genetic component (Petito et al., 2016; Sanhueza et al., 2016), as does motivation to exercise (Schutte et al., 2017). All of these findings suggest that talent is at least partially mediated by genetic factors. Indeed, it has previously been reported that ~66% of the variance in elite status is heritable (De Moor et al., 2007).

Whilst elite athlete status appears to have a strong genetic component, to date it remains apparent that the available genetic information is insufficient to reliably predict those most likely to reach elite status in the future. As discussed in the earlier part of this chapter, genetic variants most frequent in elite athletes appear to hold little to no predictive ability on their own. For example, a single nucleotide polymorphism (SNP) in *ACTN3*, a gene encoding for a protein found in fast-twitch muscle fibres, is associated with elite sprint athlete status (Yang et al., 2003). Here, between 97% and 100% of elite sprinters have at least one R allele, making the XX genotype rare in this population (Scott et al., 2010). However, the fact that at least some elite sprint and speed-power athletes have the XX genotype (Lucia et al., 2007) illustrates that it perhaps lacks the sensitivity required to correctly identify talent. In addition, approximately 80% of the world's population possess at least one R allele (North et al., 1999), thereby illustrating its lack of discriminatory power in discerning between potential athlete and non-athlete.

The inability of single SNPs to effectively discriminate between eventual phenotypes has led to the suggestion that utilising a panel of SNPs, each associated with a physical capacity deemed contributory to elite performance, may provide greater predictive ability. Using such an approach, a Total Genotype Score (TGS) is calculated, with a higher TGS indicative of a greater chance of achieving elite status. This approach has had some success, with mean TGS in athlete groups greater than controls (Ruiz et al., 2009; Ruiz et al., 2010), although it doesn't yet appear to distinguish between competitive levels within athlete groups (Santiago et al., 2010). Again, however, the sensitivity and specificity are not sufficient to rule out false positives (identifying someone as a future athlete who is later unsuccessful in this endeavor) or false negatives (identifying someone as a future non-athlete, who goes on to become a world class athlete). As such, the current consensus is that genetic testing has no role to play in the TI process (Webborn et al., 2015; Vlahovich et al., 2017a; Pickering et al., 2019b), although this opinion is formed on the assumption that elite athletes have common genotypes.

3. Is the ability to adapt to exercise a talent?

Whilst traditional TI programmes attempt to identify future elite performers through the application of physical, psychological and subjective evaluations, it's not clear whether this is the best approach. One issue with the use of such performance tests is that they measure the current status of the athlete, as opposed to the potential for that athlete to improve and develop. Consider the use of a 60m sprint test in order to identify talented sprinters in a cohort of 15-year-olds. Whilst the test is valid and will accurately identify the quickest athletes, it's not clear that the fastest athletes at age 15 will be fastest at age 25. There is, therefore, a mismatch between what the test measures—current ability—and the TI processes goal—identifying future ability (Abbott & Collins, 2002). Instead, the focus of the TI process should be to find individuals with the potential to develop their skills and physiology in order to become successful senior athletes (Abbott & Collins, 2002); commonly referred to as talent development (TD).

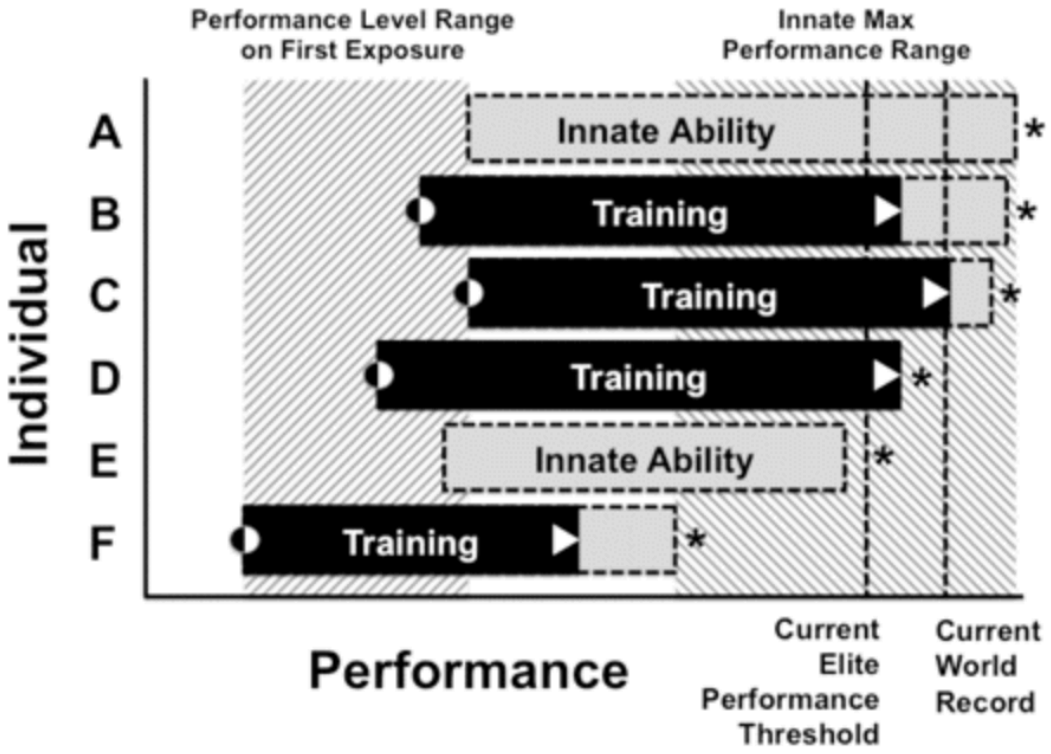

Figure 8 - A theoretical model illustrating inter-individual variation in performance and potential (reproduced from Tucker & Collins, 2012, with permission). Here, six individuals (A-F) have differing initial performance levels (performance level range on first exposure); F has the lowest, and C has the highest. The individuals also have different ceilings to his/her performance (innate max performance range), with A having the highest potential. However, training represents the journey from baseline potential to final potential; A and E do not train, and so will never reach their ceiling. Whilst C is the current world record holder, B has the potential to outperform them – but only if B can maximise their training to drive the required response.
Legend - Max = maximum; * = maximum performance threshold for each individual; triangle = current performance level; black-white circle = initial performance level.

TI programmes, therefore, should attempt to identify those with the greatest ability to develop, provided that their maximal ability is sufficient to be an elite athlete. This fits into a model proposed by Tucker and Collins (2012), detailed in figure 8 above, whereby athletes have different baseline abilities that reflect the untrained state, but also different maximal abilities, which represent the performance ceiling for each athlete. There isn't necessarily a relationship between the two; an athlete with a high start point might have a low ceiling. Conversely, an athlete with a low start point might have a higher ceiling. In this model, what becomes key is the potential of the athlete to improve with training, and whether they maximise this potential. Accordingly, for exercise adaptation to be considered a talent, it needs to fit the following five criteria proposed by Howe and colleagues (1998):

3.1. Is exercise adaptation partially innate?

An ever-increasing body of research now suggests that genetic factors modify the adaptive response to exercise. The seminal research in this regard is the HERITAGE (Health, RIsk factors, exercise Training And GEnetics) Family Study, in which sedentary adults undertook a 20-week aerobic exercise training programme. The mean post-intervention improvement in VO_{2max} in this cohort was 384 mL O_2 min^{-1}, around 25% of baseline values. However, some participants saw no improvement, whilst others exhibited much larger improvements than the mean, as high as 1100 mL O_2 min^{-1} (Bouchard & Rankinen, 2001). Genetic factors accounted for almost 50% of this inter-individual variation (Bouchard et al., 2011). Genetic association studies also show the modifying impact of single SNPs on exercise adaptation. For example, as detailed in Chapter 6, R allele carriers of *ACTN3* appear to show greater improvements in power following a strength training intervention than X allele carriers (Delmonico et al., 2007; Pereira et al., 2013). It is clear that exercise adaptation is partly genetically driven, and is therefore innate.

3.2. Are the full effects of this talent not fully evident at an early age?

Growth, maturation and the physical development of youth athletes are non-linear in nature (Abbott et al., 2005; Lloyd et al., 2016). Children and adolescents are physically less able than adult elite athletes due to differences in muscle size, strength (O'Brien et al., 2010; Waugh et al., 2013), and energy system development (Van Praagh & Dore, 2002; Ratel et al., 2006), which may limit the magnitude and type of adaptations that are possible (Pearson et al., 2006; Vaeyens et al., 2008). These developmental differences were illustrated by Radnor et al. (2017), who reported that maturation modified the adaptive response to resistance and plyometric training in a group of adolescent males. Based on these findings, it appears that knowledge of the full ability of a person to be able to adapt to exercise is likely not fully understood until maturation has occurred (Pearson et al., 2006), fulfilling this talent criterion.

3.3. Are there early indications of this talent?

This is perhaps the most difficult question to answer as part of these criteria. In part, this is due to a lack of research examining the magnitude of exercise adaptation in youths, and comparing that to either the magnitude of adaptation in those same youths as adults, or associating that adaptive response with sporting success later in life. There is a variable training response to specific training interventions in youths (Jones et al., 2016; Radnor et al., 2017), but it isn't clear how this affects adaptation in adulthood. Nevertheless, the ability to adapt favorably to exercise as a youth will positively impact development by taking the athlete from their baseline towards their performance ceiling, increasing the possibility of adult success.

3.4. Do only a minority of people possess this talent?

Overwhelmingly, research suggests that almost everyone has the ability to adapt to exercise, with the small number whom show no improvements labelled as non-responders (Timmons 2011). However, as outlined in Chapter 3, emerging research suggests such exercise non-response abates with modification of training parameters, such as an increase in training intensity (Ross et al., 2015) or frequency (Montero & Lundby, 2017). However, the magnitude of training response differs between individuals. As detailed earlier, this was apparent in HERITAGE, with a mean post-training VO_{2max} improvement of 19%, although some participants exhibited improvements of less than 5%, and others improvements of >40% (Skinner et al., 2001). Similar wide-ranging magnitudes of adaptation have been reported after strength training, and combined strength and endurance training (Hubal et al., 2005; Hautala et al., 2006; Karavirta et al., 2011). It appears that, whilst almost everyone exhibits positive adaptations to exercise, those of the greatest magnitude are limited to a smaller number of individuals; a hallmark of a talent.

3.5. Is this talent domain specific?

Whilst genetic variation exhibits a modifying effect on exercise adaptation, the final point to consider is whether this is global (i.e. all types of exercise), or modality specific (i.e. individuals exhibiting large resistance training adaptations don't necessarily exhibit the same adaptive magnitudes to aerobic training). As previously discussed, the *ACTN3* R allele is associated with greater improvements in muscle phenotype following resistance training (Delmonico et al., 2007; Pereira et al., 2013). However, regarding VO_{2max} adaptation, the X allele appears to be associated with larger improvements (Magi et al., 2016), illustrating that the genetic predisposition to exhibit a greater adaptive response is domain specific. Karavirta and colleagues (2011) randomised participants to receive strength training only, endurance training only, concurrent strength and endurance training, or no training. Within each group, participants exhibited the expected range of adaptation; however, in the concurrent training group, no subject was in the highest quintile of improvement for both VO_{2peak} and maximal voluntary contraction, again indicating that an ability to respond aerobically is separate to the ability to respond to strength training. It appears, therefore, that the ability to adapt favorably to exercise is specific to particular domains, as opposed to a global ability.

3.6. Can exercise adaptation be considered a talent?

Exercise adaptation is a highly complex and individualised process, mediated by genetic, environmental, and epigenetic factors (Pickering & Kiely, 2017a). The influence of variation at the genetic level accounting for large amounts of the inter-individual adaptive response to exercise is clear (Bouchard & Rankinen, 2001; Timmons 2011; Pickering & Kiely, 2017a), allowing the conclusion that the magnitude of adaptation is partially innate. It is also domain specific, with those possessing the ability to exhibit large improvements following one type of training not guaranteed to exhibit improvements of the same magnitude following a different modality (Karavirta et al., 2011). The presence of a small number of individuals who have very large post-training improvements in a physical trait (Skinner et al.,

2001) illustrates that only a few possess this ability. The ability to exhibit large adaptations to exercise is also potentially masked by maturation effects. So far, there is a paucity of evidence examining whether those athletes who are highly adaptable during their youth remain so during their adult years. Nevertheless, based on the evidence available, it does appear that the ability to respond favorably, and with a large magnitude, to exercise can be considered a talent.

4. Can this talent be tested for?

Traditional TI processes appear to identify athletes who are already more able than their peer group, as opposed to those who represent the greatest ability to improve. The ability to test for this latter trait would therefore enhance the TI process, providing some predictive measure as to the future level of the athlete. As Abbott & Collins (2002) state, successful prediction of future accomplishments requires identification of characteristics indicating that an individual has the potential to both develop in sport, and become a successful senior athlete. Crucially, recent research suggests that individuals respond optimally to different types of training (Beaven et al., 2008; Jones et al., 2016, Zarebska et al., 2016), illustrating that being able to match promising youngsters with the training type most likely to elicit the greatest improvements could be valuable. This can reduce the trial-and-error process, increasing the time period available for an athlete to maximise their potential by minimising ineffective and inefficient training methods.

Since the ability to respond to exercise is partially mediated by genetic factors, being able to test for these factors holds promise. A small number of studies have used this process, with early evidence suggesting they could have some predictive ability (Timmons et al., 2010; Jones et al., 2016). This process is separate from the use of genetic testing to identify the commonly held definition of sporting talent—adult performance—whereby promising athletes' genetic profiles are compared to a pool of elite athletes to look for commonalities, the assumption being that a greater number of commonalities is associated with a greater chance of being elite. At present, there is no evidence to support this (Webborn et al., 2015). Indeed, it's likely that different genes modify baseline ability (what is commonly identified in traditional TI processes) and ability to adapt to exercise, as detailed in figure 8 previously. Certainly, a greater body of research is required before evidence-based guidelines for the use of genetic testing to support talent development (as opposed to pure TI) can be utilised, but these early findings hold promise. Given the issues discussed within the current TI process, it could be argued that anything that improves the current offering should be utilised.

In addition, there are a host of ethical questions that surround genetic testing, not just within sports, but also public health (Camporesi & McNamee, 2016), some of which were discussed in detail in Part One of this chapter. The resolution of these considerations is a challenge to the translation of laboratory-based genetics research to the field, but they are related to how the information is presented and interpreted, as opposed to whether genetic information should or should not be used.

5. Conclusion

Whilst widespread across sport, traditional TI processes have a number of inherent problems. Perhaps the biggest issue is that they appear to identify current ability, as opposed to future potential, a fact which isn't helped by the poor predictive ability of currently used tests of talent. Instead, TI programmes might be better placed to identify youngsters with the greatest capacity to improve, which is partially comprised of the ability to adapt to exercise. As genetic factors account for approximately 50% of the variation in adaptation to exercise, profiling to uncover these genetic underpinnings could be a useful future adjunct to the TI process, and also allow for athletes to undertake training that they are more likely to see favorable adaptations to, creating a personalised training process making athletes more likely to achieve their potential. With the many inefficiencies and high costs associated with TI, it's clear that only marginal improvements within the TI process could make the process disproportionately more effective at developing talent, and genetic testing potentially represents this marginal gain. This section has focused on the physiological aspects of talent, and talent identification. It is, however, worth noting that sporting prowess is not dependent solely on physiology, and a number of psycho-emotional and cognitive traits are also associated with athletic achievement. Such traits include, for example, innate stress resilience, and a host of attitudinal factors, such as motivation, perseverance, and personality dispositions (Gould et al., 2007; Collins et al., 2016; Issurin 2017). Importantly, as with other phenotypes, these capacities are also partially mediated by hereditary influences, and partly by life history (Penke et al., 2007; Krueger et al., 2008). In summary, the ability to positively respond to the training stimuli imposed by physical exercise fulfils the required criteria to be considered a talent. The emergence of genetic testing may enable the more accurate identification of athletes who, thanks to a favorable genetic profile, possess a heightened ability to exhibit the greatest responses to training, thus improving the efficiency and efficacy of the talent identification process.

SECTION 4 – THE PRACTICAL USE OF GENETIC INFORMATION IN SPORT

The content of this section draws on one previously published peer-reviewed paper, and two submitted for publication, along with additional work. The published and submitted papers are:

Pickering C, Kiely J, Suraci B, Collins D. The magnitude of Yo-Yo test improvements following an aerobic training intervention are associated with total genotype score. PloS One. 2018;13(11):e0207597.

Pickering C, Kiely J. The frequency of, and attitudes towards, genetic testing amongst athletes and support staff. 2019; Under Review.

Pickering C, Kiely J. Can genetic testing predict talent? A case study of five elite athletes. 2019; Under Review.

CHAPTER 9: THE FREQUENCY OF, AND ATTITUDES TOWARDS, GENETIC TESTING AMONGST ATHLETES AND SUPPORT STAFF

Chapter preface:

Whilst there is a plethora of research exploring the impact of specific genetic variants on a variety of sporting related phenotypes, at present the extent of genetic testing within sporting contents remains poorly understood, with only one previous study (Varley et al., 2018a) attempting to quantify the true prevalence of genetic testing in high level sport. The study outlined in this chapter was undertaken in order to better understand the frequency of genetic testing in sport.

1. Introduction

Over the last few decades, research has illustrated a genetic influence on the attainment of elite athlete status (Yang et al., 2003; De Moor et al., 2007), the training-induced adaptive response (Timmons, 2001; Bouchard, 2012; Sarzynski et al., 2017), and injury risk (Goodlin et al., 2015). Recent reviews suggest that at least 155 genetic markers are associated with elite athlete status and/or fitness phenotypes (Bray et al., 2009; Ahmetov et al., 2016). As a result of these findings, a number of companies now market direct-to-consumer genetic testing to athletes and fitness enthusiasts (Webborn et al., 2015). Whilst there are vast differences in the quality of these companies, along with the validity of their claims, the current scientific consensus is that "genetic tests, based on current knowledge, have no role to play in talent identification or the individualised prescription of training to maximise performance" (Webborn et al., 2015; page 1). Similar sentiments have been echoed by the Australian Institute of Sport (Vlahovich et al., 2017a), and, furthermore, the provision of genetic testing raises a number of potentially contentious ethical issues (Camporesi & McNamee, 2016). Nevertheless, some of the authors of these consensus statements remain hopeful that the evidence-base may soon provide support for the practical use of genetic tests. Williams and colleagues (2014), for example, predicted that training modifications, both to reduce injury risk and increase training adaptations, would soon be evidence-based, and that, in the future, talent identification processes could be informed by genetic information, and these objectives represent goals of the Athlome Project Consortium (www.athlomeconsortium.org/about/). Indeed, some recent research has provided support for the contention that genetically guided training and nutritional advice for athletes may be advantageous, but more research and replication are clearly required (Jones et al., 2016; Pickering & Kiely, 2018a; Pickering et al., 2018).

Athletes and sporting teams tend to be early adopters of new technologies, as they seek innovative and novel means to gain an edge over their competitors (McNamee et al., 2018). In relation to genetic testing, this is no different; over ten years ago, the journal Nature reported that the Manly Sea Eagles, an Australian Rugby League team, had genetically tested a number of players in order to inform training programme design (Dennis, 2005). Since then, this practice has grown, with a number of sporting

teams currently known to have used the results of genetic tests in an attempt to better inform holistic athlete management and talent identification (Miller, 2016; Singer, 2017; Edwards 2018). Indeed, it was announced in 2014 that Uzbekistan's National Olympic Committee was involved in a genetic testing programme aiming to identify future elite athletes (Synovitz & Eshanova, 2014). As a result, it appears there is a mismatch between the general scientific consensus and current practice.

At present, the true prevalence of genetic testing in elite sport is largely unknown. Many organisations and/or clubs wish to retain confidentiality, potentially in part to retain an advantage over competitors, and potentially because such testing may be negatively received by the public and media. Recently, Varley and colleagues (2018a) conducted an online survey of 72 elite athletes and 95 support staff based within the UK. Their results indicated that fewer than 17% of elite athletes had undergone a genetic test, although most athletes and coaches (79%) indicated that they were willing to engage in such tests. However, in that online survey, respondent numbers were somewhat limited, and the diversity of sports represented was low. In directly addressing this information deficit, the present study was designed to a) determine the prevalence of genetic testing in sports, and b) advance understanding of the relevant prevailing beliefs and opinions of athletes, sports coaches, sports scientists, and sports medicine providers, as to the utility of genetic testing in sports. In addition, the secondary aims were to a) determine whether teams or individuals who had utilised genetic testing found the acquired information relevant and useful, and b) identify the perceived barriers amongst athletes and support staff towards genetic testing.

2. Methods

Prior to the commencement of data collection, ethical approval was granted by the University of Central Lancashire Ethics Board in accordance with the Declaration of Helsinki. Athletes and support staff were recruited via social media accounts from both the thesis author and his supervisor. Potential participants were provided with a link, which directed to the survey home page; this page contained both the participant information sheet and informed consent form. The participants were asked to provide informed consent, and complete an online survey related to both their views and use of genetic testing within sport. The survey was comprised of 42 questions, with participants directed to specific questions based on their previous answers. The majority of questions were multiple choice, although two required a written answer. The questions broadly fit into four groups: 1) demographic data, 2) beliefs about the effects of genetics in sport, 3) prevalence of genetic testing in sport, and 4) the utility of genetic testing in sport. The questionnaire can be found in full in the appendix. Following the completion of the questionnaire, frequency-based descriptive analysis was carried out.

3. Results

3.1 Participant demographics

Two hundred and fifty-six individuals gave consent to take part in this study and completed the survey, comprising of 110 current or former athletes (45.3%) and 133 members of support staff (54.7%). The majority of respondents (76.5% of athletes and 84% of support staff) were male. The most common sport, for both athletes (66%) and support staff (40%), was athletics. Table 5 below lists the sports with the frequency of respondents.

Primary Sport	**Athlete [n, (%)]**	**Support Staff [n, (%)]**
Athletics	73 (66%)	53 (40%)
Football	4 (3%)	17 (13%)
Rugby (League/Union)	2 (2%)	20 (15%)
American Football	4 (3%)	3 (2.5%)
Basketball	0 (0%)	5 (4%)
Swimming	1 (1%)	3 (2%)
Racquet Sports	0 (0%)	3 (2%)
Winter Olympic Sports	7 (7%)	4 (3%)
Other	19 (18%)	25 (19%)

Table 5 – Frequency of different sports within survey sample

Sports in the "other" category included rowing (4% of athletes; 2% support staff), combat sports (4% of athletes; 3% support staff), volleyball (1% of athletes & support staff), field hockey (1% of athletes & support staff), cycling (4% of athletes & support staff), and triathlon (1% of athletes).

18% of the athletes taking part in this survey had competed at the Olympic Games or World Championships, and a total of 51% had represented their country. A further 22% had competed at the highest level within their country, such as the national championships or top league. The vast majority (78%) were from the UK and Ireland; 9% were from the US, and 6% from other European Countries.

Within the support staff cohort, 18% most frequently worked with an Olympic or World Championships competitor, with 36% of respondents in total working with international athletes, and a further 30% working with athletes who had competed at the highest level within their country. Most of the support staff (53%) were sports coaches, 18% were strength and conditioning coaches, 12% were sports scientists, and 5% were physiotherapists. Most (62.5%) were from the UK and Ireland, with a further 11% from other European countries, 7.5% from the US, and 5% each from Australia & New Zealand and North America (excluding the US).

3.2 Beliefs around the impact of genetics on sporting phenotypes

Participants were asked about their opinion as to the relative contribution of genetics to various sporting phenotypes. These results are shown in table 6.

		Athletes	**Support Staff**
What impact do you think an individual's genetic make-up has on their chances of being an elite athlete?	None	2%	1%
	Minimal (<25%)	6%	8%
	Somewhat (25-75%)	59%	69%
	Almost Entirely (75%+)	33%	22%
What impact do you think an individual's genetic make-up has on their sporting/fitness improvements following exercise?	None	3%	2%
	Minimal (<25%)	9%	7%
	Somewhat (25-75%)	59%	71%
	Almost Entirely (75%+)	29%	21%
What impact do you think an individual's genetic make-up has on their nutrition requirements?	None	2%	3%
	Minimal (<25%)	23%	27%
	Somewhat (25-75%)	57%	59%
	Almost Entirely (75%+)	18%	11%

Table 6 – Athlete and Support Staff opinions as to the impact of genetics on sporting phenotypes.

These beliefs differed slightly between individual and team sport athletes. A greater proportion of individual sport athletes (38%) believed that an individual's chance of becoming an elite athlete was almost entirely down to genetic make-up, as opposed to just 18% of team sport athletes. Outside of this question, beliefs around the extent of genetic variation on training response and nutritional requirements were similar between team and individual sport athletes.

3.3 Prevalence of genetic testing within sport

10% of the athletes had utilised a genetic test that was targeted at sports performance, and 12% of support staff respondents stated that they had utilised genetic testing within their organisation. The prevalence differed slightly between team and individual sport athletes, with 8% of individual team sport athletes reporting having undertaken a genetic test, compared to 17% of team sport athletes.

3.4 Attitudes towards genetic testing

The 90% of athletes and 88% of support staff respondents who had not utilised genetic testing were asked for their reasons for having not done so. These results are reported in table 7.

	Athlete	**Support Staff**
Too expensive	31%	41%
Unaware that genetic testing was possible	49%	25%
Insufficient evidence for its use	21%	39%
Concerns around data protection	1%	6%
Concerns about negative press coverage	0%	2%
Ethical considerations	4%	19%

Table 7 – Most common reasons cited for not utilising genetic testing

Of those who had not utilised genetic testing, 10% of athletes and 5% of support staff envisioned doing so within the next year, 26% of athletes and 28% of support staff within the next 5 years, and 11% of athletes and 29% of support staff within the next 10 years. 53% of athletes and 38% of support staff believed they would never utilise genetic testing. Again, there was minimal difference between individual and team sport athletes in this regard; 56% of individual and 44% of team sport athletes said they envisaged never undertaking a genetic test, 25% (individual) and 28% (team) believed they would in the next 5 years, and 11% (both) believed they would within the next 5-10 years. Perhaps the main difference between the two sporting types was in the proportion stating they would take a genetic test within the next year; 8% of individual compared to 17% of team sport athletes. Table 8 (below) provides the most frequent responses to the question “what would cause you to use genetic testing?”

	Athletes	Support Staff
Publication of peer-reviewed case studies	29%	63%
Greater number of intervention studies	14%	36%
Publication of randomised controlled trials	26%	46%
More athletes/teams using it	48%	18%
Players requesting it	N/A	25%
Direct approach from genetic testing company	N/A	18%
Increased awareness of the product	35%	N/A
Lower price	48%	36%

Table 8 – Responses to the question "what would cause you to use genetic testing?"

3.5 Experience of genetic testing

Of the athletes who had utilised genetic testing, the most common reason cited (44%) was to inform training programme design, along with general interest (22%), to identify the best sport to compete in (11%), and injury prevention and nutritional insights (11%). 78% of athletes who had undertaken a genetic test reported that the information they gleaned from it was useful. Of the 22% who did not find the information useful, the main reason (100%) was that the information provided was too generic, and not targeted at sports people (50%). Most athletes (75%) found that the results of their genetic test were easy to understand, with 75% receiving after-testing follow-ups from the testing company to provide them with additional information. The majority of athletes (75%) who had undertaken genetic testing reported that they had made changes based on the results of the test.

Similarly, of the support staff members that had utilised genetic testing within their organisation, 50% had done so primarily to inform training programme design, 21% for injury prevention, and 15% to guide nutritional interventions. Interestingly, 7% had done so as a screen for disease risk, and none did so as a talent identification tool. 60% of support staff who had used a genetic test found the information useful; of those who didn't, the main reason (80%) was that the results were too generic. Most (85%) found the information provided easy to understand, and 65% received follow up information from the testing company/institution. 65% of support staff who had utilised a genetic test within their organisation made changes based on the results of the test, with 100% of respondents stating they made changes to their athletes training programme, 80% to their diet, 67% to their recovery, and 40% to their lifestyle.

All of the genetic testing reported by athletes in this study was conducted by a commercial company. Conversely, for support staff respondents, 15% of the genetic testing had been carried out by a university or academic institution, with the remaining 85% coming from commercial companies.

4 Discussion

This study, which surveyed high level athletes and support staff from across the globe, suggests that genetic testing in elite sport remains infrequent and sporadic, with only 10% of athletes and 12% of support staff who responded to this survey stating that they utilised genetic testing within their practice. This prevalence of genetic testing in athletes is similar to previously published research (Varley et al., 2018a), although the reported use by sporting organisations was much higher (12%, compared to 2% in Varley et al., 2018a). The present study builds on previous research by Varley and colleagues (2018a) by increasing the sample size of athlete and support staff, from 167 (72 athletes and 95 support staff) in Varley and colleagues (2018a) to over 400 in total. This increases the robustness of the findings of both studies. Additionally, the majority of athletes surveyed in Varley and colleagues (2018a) were from the sports of rugby, speed skating, and volleyball. In comparison, the majority (66%) of athlete respondents in the present study were from the sport of athletics; as a result, the present study serves to add respondents from a greater range of sports to the evidence base.

Overall, the survey respondents believed that genetics has a sizeable (>25%) impact on an individual's potential to be an elite athlete (92% of athletes and 91% of support staff). These attitudes correspond to the findings of published research exploring the genetic influence on sporting phenotypes. For example, De Moor and colleagues (2007) reported that heritable factors explained approximately 66% of the variance in elite athlete status between individuals. Furthermore, a number of single nucleotide polymorphisms have been identified which may increase an individual's chance of attaining elite athlete status (Ahmetov et al., 2016), although the research in this area remains equivocal (Rankinen et al., 2016). However, at present, this information does not appear to be all that useful in identifying potential elite athletes (see Chapters 8 and 12 for further details), leading to the general scientific consensus being that genetic testing should not be used as a talent identification tool (Webborn et al., 2015; Vlahovich et al., 2017a). This viewpoint appears to be mirrored in the practice of support staff, of which none had utilised genetic testing as a talent screen. One athlete did, however, report using their genetic results as a means of identifying which sport they should compete in. Interestingly, individual athletes were more likely to report that they believed genetic variation had a considerable (>75%) effect on the chances of becoming an elite athlete than team sport athletes, although the reasons for this are currently unclear.

Additionally, 88% of athletes and 93% of support staff respondents believed that genetics has a sizeable (>25%) impact on an individual's improvements following a training programme. Again, this is mirrored in the research literature; individual SNPs, such as *ACTN3* and *PPARGC1A*, appear to modify the magnitude of post-training adaptations (Chapter 6; Ring-Dimitriou et al., 2014). More recently, studies have started to explore the utility of Total Genotype Scores in explaining the variation in training response (Chapter 10; Moraes et al., 2018; He et al., 2018), and potentially in maximising the adaptations to exercise (Jones et al., 2016). Of the athletes within this study who indicated they had undertaken genetic testing, 44% stated it was to inform training programme design, as did 50% of support staff.

Interestingly, fewer athletes (74%) and support staff (70%) believed that genetics had a sizeable (>25%) impact on an individual's nutrient requirements. This is somewhat surprising, given that the field of nutrigenetics is well established, with a number of studies demonstrating how SNPs in genes such as *MTHFR* (Ashfield-Watt et al., 2002) and *SOD2* (Li et al., 2005) can potentially modify micronutrient status and requirements, although this may be outside the scope of practice of many involved within elite sport. Only 11% of athletes, and 15% of support staff, utilised genetic testing to gain insights into nutritional requirements.

Although the vast majority of both athletes and support staff surveyed believed that genetics had a substantial influence on a number of sporting phenotypes, the overall uptake of testing was somewhat low (~10%). This study explored some potential reasons for this disparity. 49% of athletes stated they were unaware that genetic testing was available, suggesting that one of the main drivers for a lack of uptake of genetic testing is due to awareness. A greater number of support staff were aware that genetic testing was available, possibly due to the various recent publications in the scientific literature on the subject (e.g. Webborn et al., 2015; Jones et al., 2016). However, potentially due to the conclusions of two recent consensus statements (Webborn et al., 2015; Vlahovich et al., 2017a), a large proportion (39%) of support staff believed that there was insufficient evidence for the use of genetic testing within elite sport. Cost was also an issue, with 31% of athletes and 41% of support staff stating that one the reasons they had not utilised genetic testing was that it was too expensive. Whilst, historically, genetic testing has been costly (Hayden, 2014), in recent years technological improvements and increased sales volumes have reduced prices, such that a genetic test today typically costs £100-£200. Neither athletes nor support staff appeared especially concerned around data protection or negative press coverage, with few citing these as reasons they had not undertaken genetic testing. However, 19% of support staff stated that ethical considerations, such as the perceived use of genetic information for talent identification, were one of the reasons they had not utilised genetic testing.

Support staff generally noted a need for increased scientific evidence before they would consider utilising genetic information in the future. Conversely, athletes were less concerned about this, instead stating that, if more athletes and sports teams began using genetic testing, they too would consider it. Both athletes (48%) and support staff (36%) stated that a reduction in price would lead them to consider genetic testing, and 25% of support staff would consider a genetic test should a player request it. This latter finding is interesting, as it demonstrates that many practitioners understand the value of player buy-in and potential expectancy effects surrounding the use of genetic information, similar to that found in a survey of Premier League medical staff regarding the use of Platelet Rich Plasma injections (McNamee et al., 2018).

When genetic testing had been used, athletes (78%) and support staff (60%) tended to perceive it to be useful. The main reasons cited for a lack of utility were that the information was either too generic, or not targeted specifically towards sports people. Most athletes who had undertaken a genetic test stated that they had made changes to either their training or lifestyle based on the results of the test, demonstrating a perceived utility of the genetic information. The vast majority of genetic testing reported

by participants in this survey was carried out by commercial companies, as opposed to academic institutions. Most athletes (75%) and support staff (65%) received follow up information from the testing company, giving them the opportunity to ask additional questions and clarify any misunderstandings.

4.1 Implications for future work

Whilst not hugely prevalent as of yet, the results of this current survey, and previous work (Varley et al., 2018a), suggest there is an appetite for genetic information within elite sport. The use of such information brings with it a host of ethical considerations. Many of these have already been identified in previous chapters, but are worth repeating. For example, is it ever ethical to test those under-18, who, in theory, cannot provide informed consent? Can a sporting organisation recommend or even require genetic testing of its athletes, and at what point does this constitute coercion? Who owns the genetic data, and where and how is it stored? What happens to this data if a player leaves a club/organisation, or retires? What provisions, if any, are made for the discovery of potentially disease-causing or disease-associated variants within an athlete's genetic data? How would this discovery affect relatives of the athlete, who may also require genetic screening for the particular disease variant? What are the additional healthcare burdens and costs associated with this? At present, there are no guidelines assisting practitioners in answering these questions, or even guiding them towards an informed decision. As such, the development of such guidelines represents a potential opportunity to enhance practice. This is potentially of significant importance, given that one respondent to the present survey stated that they had utilised genetic testing within their organisation to screen for disease risk; it's not clear how they used this information, nor how it may have been communicated to the athlete(s) in question.

Furthermore, a consistent theme throughout the early part of this thesis (see Chapter 4) is that, at present, genetic information assists practitioners in *explaining* what has already happened, but does not assist them in *predicting* a future outcome, which, in elite sport, is of greater importance; a coach does not necessarily need to explain why a previous training programme was ineffective, but rather prevent the athlete from undertaking ineffective training in the future. As such, future research in this area should explore the use of a wider number of genetic variants, and aim to assist athletes and practitioners in designing better training programmes. This would overcome one of the major barriers for the use of genetic information in elite sport, which is a perceived lack of evidence of utility.

4.2 Limitations

Whilst the results of this survey are novel and interesting, with the potential to impact research and practice, there are some potential limitations. Firstly, the survey was shared the thesis author and supervisor on social media. Given the author's employment status at that time—as Head of Sports Science at a genetic testing company—it is possible that individuals following him (and hence being more likely to have seen the survey link) have an increased interest in genetic testing, and may not be representative of athletes and support staff in general. However, the extent of athletes stating they had undertaken genetic testing in this study (10%) was actually lower than in previous research (Varley et al.,

2018a), suggesting this had not skewed the results. Additionally, the vast majority (~80%) of respondents were male; whilst there is no apparent reason why females would be more or less likely to undertake a genetic test, or hold different attitudes towards such a test, this possibility cannot be excluded. Furthermore, the questionnaire utilised in this study was not validated, was not tested for face validity or internal reliability, and did not undergo principal component analysis. Whilst this is a limitation, the study was designed to build upon the work of Varley and colleagues (2018a), whose questionnaire was also not validated, but still provided interesting data on a very under-researched topic. Finally, the survey was undertaken anonymously; as a result, it is possible that the support staff survey respondents were from the same club or organisation, artificially inflating the apparent prevalence of genetic testing based on the results of these respondents. Future research should address these limitations by aiming to recruit a higher number of female athletes—the lack of female support staff participants may (sadly) mirror actual female representation within high-level sport (Kane & LaVoi, 2017)—and verify that only a single support staff responds from each club or organisation. Future studies should also aim to recruit an increased number of participants in sports inadequately covered here, and explore the attitudes towards genetic testing within regions that were not adequately covered here. That said, the present study recruited a number of Olympic/World Championship athletes (18%), with over half the sample being international athletes. Additionally, of the support staff polled, 36% stated that their most frequent contact was with athletes of an international standard, demonstrating that the cohort was of a high standard, a potential strength of this study.

5. Conclusion

Despite the fact that the majority of athletes and support staff polled in this survey stating their belief that genetics exert a sizeable influence on a number of sporting-related traits, the overall uptake of genetic testing within this cohort, in which more than half of the athletes polled had represented their country, was low, at around 10%. The prevalence of genetic testing is similar to that previously reported (Varley et al., 2018a). The reasons for this relatively low uptake are varied, but include a lack of awareness, cost, and a lack of scientific evidence underpinning the use of such tests. Despite concerns from researchers in this field (Webborn et al., 2015), it appears that the vast majority of those who have utilised genetic testing within sport are not doing so as a talent identification tool, and instead are doing so as a method to inform training programme design. Whilst there is some evidence supporting the use of genetic information in this way (Jones et al., 2018; Chapter 6-8), additional research is required to build an evidence-based framework for the use of genetic information within sport, along with the development of best-practice guidelines regarding the testing of athletes by a sporting organisation, including how an individual's data can best be shared with clubs/organisations.

CHAPTER 10: THE MAGNITUDE OF YO-YO TEST IMPROVEMENTS FOLLOWING AN AEROBIC TRAINING INTERVENTION ARE ASSOCIATED WITH TOTAL GENOTYPE SCORE

Chapter preface:

As identified in previous chapters, the majority of exercise genetics research tends to focus on exploring the effect of single genetic variants on an outcome, such as the attainment of elite athlete status, or the magnitude of training adaptations. However, this information is potentially of limited use to practitioners and athletes in the field, who want information that can inform training programme design. Furthermore, individual genes are likely to have very poor explanatory or predictive capabilities in isolation. Consequently, Total Genotype Scores (TGS), where a number of genetic variants are pooled together, are being increasingly used to increase the predictive ability of a genetic test. The study outlined in this chapter, which was published in *PLoS One* (Pickering et al., 2018), is the first to utilise a commercially available TGS, in this case comprised of 5 SNPs, in the prediction of the magnitude of improvements in Yo-Yo test following a standardised training intervention.

1. Introduction

Aerobic capacity (as determined by maximal oxygen uptake, VO_{2max}) is considered crucial for sports performance. The greater the aerobic capacity of an athlete, the longer they can exercise at a given intensity (Jones and Carter, 2000). Additionally, aerobic fitness enhances recovery from high intensity intermittent exercise, such as that found in most team sports (Tomlin and Wenger, 2001), and also potentially differentiates between performance levels, with elite team-sport athletes scoring higher than their sub-elite and amateur counterparts on tests of aerobic fitness (Bangsbo et al., 2008; Tønnessen et al., 2013). Furthermore, improvements in aerobic fitness following training have been associated with improvements in soccer performance (Helgerud et al., 2001). As such, aerobic fitness training is a fundamental inclusion in most professional team-sport physical preparation programmes.

Similarly, within endurance sport training there is on-going debate, in both the academic and coaching domains, focused on uncovering the "best" combination of running volumes and intensities necessary to optimally drive positive adaptation, and hence improve performance (Seiler et al., 2013). However, the belief that there is a universal "best" type of training to develop aerobic performance is predicated on the implicit assumption that athletes respond to the imposed training demands in a broadly similar fashion. In recent years, this conventional presumption has been challenged by empirical evidence showing unexpectedly extensive inter-individual variation in aerobic fitness gains experienced by participants undertaking identical training interventions (Bonafiglia et al., 2016; Bouchard et al., 1999; Ross et al., 2015; Scharhag-Rosenberger et al., 2012). This inter-individual response diversity is exemplified by the collection of studies constituting the HERITAGE (HEalth, RIsk factors, exercise Training And GEnetics) Family Study; whilst the mean improvement in aerobic fitness following training was 19%, some participants saw improvements as high as 40%, whilst others experienced no

improvements (Bouchard et al., 1999). Further analysis of the HERITAGE data revealed that genetic variation between participants explained approximately 47% of this variance (Bouchard et al., 1999), although this data has recently been critically evaluated (Williamson et al., 2017). Such extensive inter-individual variability has been replicated in a number of other studies examining adaptations to aerobic training (Bonafiglia et al., 2016; Ross et al., 2015; Scharhag-Rosenberger et al., 2012).

The demonstrated magnitude of the inter-individual adaptive response following aerobic training poses a potential problem to conventional exercise prescription methodologies. For example, professional athletes may fail to elicit expected benefits, and patients prescribed aerobic exercise—under the premise that such training will improve health parameters—may fail to realise meaningful benefits, despite engaging in the recommended training. Since the completion of the HERITAGE Family Study, the field of sports genetics has grown exponentially. Currently, 155 genetic markers are associated with elite athlete status (Ahmetov et al., 2016), and more still are associated with training response (Bray et al., 2009). However, the translation and application of this research to both sports training and general health contexts remains both tentative and controversial (Webborn et al., 2015).

Previously, research has focused on exploring the influence of genetic variations on elite endurance athlete status, with a general lack of predictive ability (Yvert et al., 2016; Rankinen et al., 2016). However, with heritable factors potentially accounting for close to half of the variation in exercise response between individuals (Bouchard et al., 1999), there is the potential that insight into the genetic profile of the individual could improve exercise programme design. Research on the impact of genetic variation on exercise adaptation has identified a series of single nucleotide polymorphisms (SNPs) which may contribute to observed differences in response to aerobic training. Five of these SNPs from four different genes (*VEGF* [Ahmetov et al., 2008], *PPARGC1A* [Ring-Dimitriou et al., 2004], *CRP* [Kuo et al., 2007b; Obisesan et al., 2004], and two from *ADRB2*[Moore et al., 2001; Wolfarth et al., 2007; Sarpeshkar and Bentley, 2010]) have been collated into an algorithm used in a commercially available test. These SNPs affect different dimensions of cardiovascular function, and are associated with either VO_{2max} scores, or improvements in this capacity following aerobic training.

Given the observable inter-subject variations in training-induced aerobic adaptations, the ability to identify individuals who may exhibit smaller fitness gains could enable the evolution of more personalised training programme designs. Such an innovation would promote greater overall improvements within populations, enhancing training efficiency and increasing the chances of positive adaptation in a greater number of individuals. Therefore, the purpose of this study was to determine whether a commercially available genetic algorithm was associated with the magnitude of improvements in aerobic fitness in a group of youth soccer players following an eight-week training block. It is believed that players with a greater number of positive alleles for genes associated with higher aerobic fitness would see larger improvements following aerobic training than those with fewer positive alleles. A secondary aim is to attempt to bridge the gap between genetics research and sports science practice. The ability to utilise genotype assessment panels to inform training programme design holds the potential to revolutionise exercise prescription in medical, health and sporting domains. Yet genetic research, whilst potentially impactful, can often appear confusing to field-based practitioners and athletes, who require

real-world data to inform their decision-making processes (Buchheit, 2017). Accordingly, this work is framed as a training observation study, as opposed to a genetic association study. The outcomes may provide meaningful, actionable training insights promoting the strategic incorporation of genetic information into training programme designs.

2. Methods

2.1 Participants

Following University of Central Lancashire Ethics Committee approval according to the Declaration of Helsinki, a convenience sample of 42 male soccer players aged between 16-19 years of age (height 176 ± 6 cm, weight 69 ± 9 kg) from a college soccer academy volunteered to participate in this study. The sample was chosen to best represent the size of a typical soccer squad. Each player had an average of 11 years' football training experience, and was actively competing in the English College Football Association Leagues.

2.2 Methodology

Participants were in a phase of training aimed at increasing aerobic capacity via sport specific conditioning, in this case small sided games. Sessions took place twice a week for the eight-week training block. Within each session, the participants undertook small-sided games on pitches of differing sizes and with a different number of players, ranging from 3 v 3 to 5 v 5. The work periods were uniform in all sessions, consisting of four sets of four-minutes exercise and three-minutes of active recovery. Small-sided games have previously been demonstrated to be an effective method of enhancing aerobic fitness in soccer players (Impellizzeri et al., 2006; Hill-Haas, 2009; Dellal et al., 2012; Radziminski et al., 2013; Clemente et al., 2014), and, as they also enhance sport-specific technical and tactical skill (Radziminski et al., 2013), they represent a preferred method of player development to many soccer coaches (Clemente et al., 2014). A recent meta-analysis (Hammami et al., 2018), across all team sports, reported a large beneficial effect (ES = 1.94) of small-sided games on VO_{2max} fitness improvements in team sport players, suggesting that this training method is an effective means of enhancing the physiological capabilities of soccer players. An eight-week study period was utilised as this has previously been shown to be a sufficient period of time to elicit aerobic and other performance improvements following the use of small-sided games (Impellizzeri et al., 2006; Radziminski et al., 2013), with most small-sided games studies utilising intervention periods of 6-8 weeks (Hammami et al., 2018). The training protocol of four sets of four-minute exercise bouts, interspersed with three-minutes of active recovery was selected based off current best practice guidelines (Clemente et al., 2014). All sessions were supervised by a UEFA A Licensed coach, who set and monitored the intensity of each training session, through the use of Rating of Perceived Exertion (RPE). The participants were also taking part in a minimum of one competitive match per week during this time. No additional training was prescribed during the intervention period. There was no control group, as requesting a group of competitive footballers to refrain from exercise is potentially in violation of the Declaration of Helsinki, and is almost certainly unethical (Shepherd 2001).

Before and after the training block, participants' aerobic fitness was assessed by the Yo-Yo Intermittent Recovery Test, level 1 (Yo-Yo IR1), a reliable and valid measure of aerobic fitness (Krutstrup et al., 2003). Briefly, the test is comprised of repeated 2 x 20 m runs back-and-forth performed to an audible beep, separated by an active rest period of 10 seconds. The time allowed for each 20 m section decreases as the test progresses, resulting in a faster required running speed; this begins at 10 $km \cdot h^{-1}$, and is increased by 2 and then 1 $km \cdot h^{-1}$ for the respective next two speed levels. After this, the speed increases by 0.5 $km \cdot h^{-1}$ for each additional level. The test is halted when a participant fails to cover the distance in the required time on two consecutive occasions, indicating that exhaustion has occurred. All participants were provided with verbal encouragement during the test. Participants refrained from caffeine for at least 12 hours before testing, which took place outdoors on a soccer pitch, at the same time of day on both occasions. Individual results were expressed as distance covered in metres. Participants had carried out Yo-Yo tests previously, and were fully accustomed to the assessment protocol.

2.3 Genetic testing

Alongside the training programme, participants underwent genetic testing using a commercially available self-testing kit from DNAFit Life Sciences. Participants provided a saliva sample, collected using a sterile buccal swab. The samples were sent to IDna Genetics Laboratory (Norwich, UK), where DNA was extracted and purified using the Isohelix Buccalyse DNA extraction kit BEK-50 (Kent, UK), and amplified through PCR on an ABI7900 real-time thermocycler (Applied Biosystem, Waltham, USA). Through this process, genetic information regarding SNPs believed to affect aerobic trainability (*VEGF* rs2010963, *ADRB2* rs1042713 and rs1042714, *CRP* rs1205 & *PPARGC1A* rs8192678) (Ahmetov et al., 2008; Kuo et al., 2007b; Obisesan et al., 2004; Ring-Dimitriou et al., 2004; Moore et al., 2001; Wolfarth et al., 2007; Sarpeshkar and Bentley, 2010) was determined. Each allele was given a score of between 0 and 4 points depending on the expected magnitude of its effects on improvements in aerobic fitness following training. The strength of the rating was based on the evidence from cumulative literature results averaged over time. The sum of these points was combined to give an overall score. This method is identical to Jones et al. (2016), and similar to the methods used in other studies utilising genetic algorithms (Meckel et al., 2014; Ruiz et al., 2009). The participants were stratified into three groups; "low", "medium" and "high" depending on their weighted total genotype score (TGS), with a higher score indicating possession of a greater number of alleles expected to improve adaptation to aerobic training. Those with an overall score of 40% or less were classed as "low". Scores of 41-70% were classed as "medium". A score of >70% was classed as "high". These divisions were used in the absence of previous work, and represents a gross sub-division into categories based on the expectation that approximately 60% of individuals have a score of between 40-70% (Pickering, https://blog.dnafit.com/am-i-normal-aerobic-trainability). The divisions used here mirror those utilised by DNAFit Life Sciences in their commercially available genetic test; as discussed in Chapter 4 (Methodology), a main aim of the present thesis is to explore the utility of commercially available tests in the athlete preparation process. All athletes were blinded to their genetic results until completion of the final testing.

2.4 Statistical analysis

Means, standard deviations and 90% confidence intervals (CI) were calculated for whole group and sub-groups for both pre- and post-training test scores. 90% CI were used as per the recommendations of Sterne and Smith (2001) and Hopkins et al., (2009). These were examined by a 3 X 2 (Group X Time) mixed methods ANOVA, with repeated measures on the second factor. The dependent variable was the Yo-Yo score (pre- and post-) obtained by each participant. Tukey's HSD was also run. To further discover the differences between groups, pre- and post-training test scores were compared within groups using a paired sample t-test, and between groups using unpaired t-tests. Statistical significance was set as $P \leq 0.05$, which after adjustment using Bonferroni correction led to a significance level of 0.008 for the six t-tests. Cohen's d was calculated for within- and between-group effect size. The thresholds used were <0.2 (trivial), 0.21 - 0.5 (small), 0.51 - 0.8 (moderate), 0.81 – 1.2 (large), 1.21 – 2 (very large), >2 (huge) (Cohen 1988; Sawilowsky 2009). Data were analysed using Microsoft Excel 15.29 (Microsoft Corporation, Redmond, WA, USA) and IBM SPSS Statistics 23 (IBM Corporation, Armonk, NY, USA).

3. Results

Table 9 illustrates the genotype-group data. After examination with a 3 X 2 (Group X Time) mixed methods ANOVA, there was a significant main effect of time ($F(1, 39) = 67.8$, $P < 0.001$) and a significant interaction ($F(2, 39) = 10.9$, $P < 0.001$). The main effect of Group ($F(1,39) = 5.11$) was not significant.

The significant main effects of Time support the impact of the aerobic training intervention, as all groups showed an improvement in fitness. In contrast, follow up on the between group main effect using Tukey's HSD showed no significant differences (all pairwise comparisons non-significant). As such, groups were taken as being equivalently fit at baseline.

The interaction effects were of most interest, in that these addressed the main purpose of the study. Building on the significant overall differences demonstrated by the significant interaction, follow-up was conducted by use of three paired t-tests on the before and after data of the three groups. These results are shown in table 9.

Group	Pre-training Yo-Yo Score (m) [mean (SD; 90% CI)]	Post-training Yo-Yo Score (m) [mean (SD; 90% CI)]	P-Value for Difference Between Pre- and Post-training Scores (paired t-test)	Effect Size (Cohen's d) (90% CI)
Low (n = 6)	1006 (292; 766 to 1247)	1073 (281; 842 to 1304)	0.0041	0.23 "Small"
Medium (n = 23)	1045 (472; 876 to 1213)	1409 (453; 1246 to 1571)	<0.0001	0.79 "Moderate"
High (n = 13)	969 (493; 725 to 1212)	1529 (508; 1278 to 1780)	<0.0001	1.12 "Large"

Table 9 – Pre- and post-training Yo-Yo test scores, stratified for individual genotype groups.

The data for between-group interactions was then analysed, and is summarised in figure 9. The key finding is that there was a significant difference ($P < 0.05$) between all groups, which remained after Bonferroni correction for differences between "low" and "high", and "low" and "medium" comparisons. The effect sizes were very large (1.32) for the difference between "low" and "medium" groups, large (0.82) for differences between "medium" and "high", and huge (2.59) for differences between "low" and "high" groups.

In all groups, the mean improvement was 382 ± 270 m (90% CI 312 to 452 m), which represents an improvement of 37.5%. Within the "low" group, the mean improvement was 67 ± 33 m (90% CI 40 to 94 m), representing a mean improvement of 7.5%. No participant in the "low" group exhibited an improvement greater than 120 m. In the "medium" group, the mean improvement was 364 ± 248 m (90% CI 274 to 452 m), representing a mean improvement of 43.8%. Within this group, two participants exhibited a negative improvement (i.e. got worse), whilst all other participants (21/23; 91%) showed improvements greater than 120 m. Five participants (22%) from the "medium" group showed an improvement of greater than 500 m. In the "high" group, the mean improvement was 560 ± 225 m (90% CI 449 to 671 m), representing a mean percentage improvement of 72.6%. In the "high" group, 9/13 (69%) of participants demonstrated an improvement of greater than 500 m, with all participants (100%) showing an improvement of 120 m or greater. There was considerable inter-individual variation in magnitude of aerobic improvements between participants, as illustrated in figure 10.

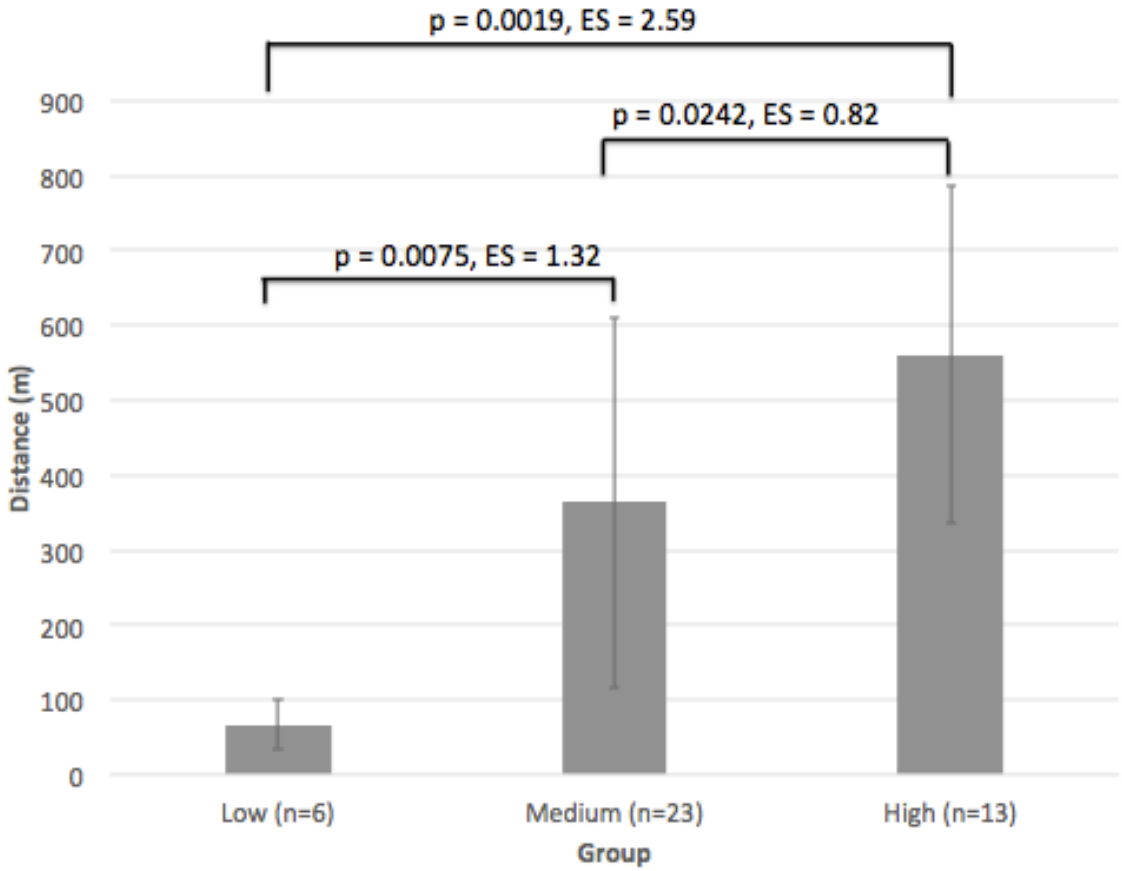

Figure 9 – Between group interactions for post-training improvements in Yo-Yo Score.

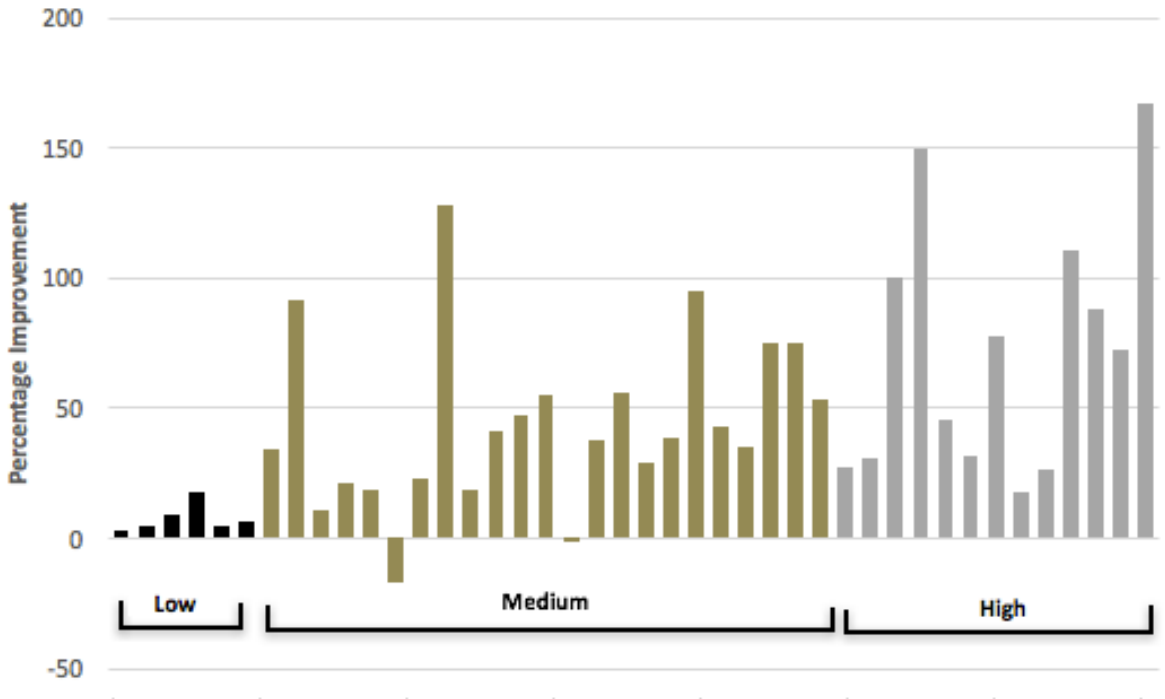

Figure 10 – Individual percentage improvement scores across “low”, “medium” and “high” groups.

4. Discussion

The results of this study indicate that, following an eight-week training period, the magnitude of improvements in Yo-Yo test scores show significant inter-subject variation. This finding is in agreement with previous research examining variability in aerobic fitness improvements following training (Bouchard et al., 1999; Timmons et al., 2010). Crucially, the magnitude of training improvements was associated with a five SNP TGS determined by genetic profiling before training began.

The use of this genetic algorithm did not predict absolute performance in the Yo-Yo test. This observation adds to previous work suggesting that genetic testing should not be used as a talent identification tool (Webborn et al., 2015). However, the results of the algorithm were associated with the magnitude of improvements in Yo-Yo score following training. To illustrate how this algorithm does not predict aerobic "talent", the lowest pre-training (440 m) and post-training (640 m) score occurred within a participant from the "high" genotype group. If genetic tests were to have utility in the prediction of talent, it would be expected that the lowest aerobic test scores would occur in the "low" group. However, this same participant's test improvement (200 m) was greater than every participant in the "low" group. This supports the assertion that the genetic-based algorithm has utility in predicting training response, not talent. Similarly, when the two participants who exhibited a reduction in Yo-Yo score in the post-training test are removed, every participant from the "medium" and "high" group showed improvements equal to (n = 1) or greater than (n = 25) those in the "low" group. Of the two participants exhibiting lower post-training scores, one had a score reduction of 40m (from 2440 m to 2400 m), a 1.64% reduction, which is within the range of Yo-Yo test-retest variation previously reported (Krustrup et al., 2003). The second participant had a performance decrement of 240 m; which, whilst substantial, remains unexplained.

The potential to predict response to aerobic training may be useful to ensure that appropriately individualised training methods are utilised to maximise training adaptations. For example, if an individual is classed as having a low aerobic trainability, it might be prudent for them to follow a different training programme to an individual classed as having a high aerobic trainability. There are many ways to increase performance in aerobic endurance activities, including improvements in VO_{2max}, running economy, lactate threshold, and VO_2 kinetics (Jones and DiMenna, 2011). In individuals with a low aerobic trainability, diverting training resources towards optimising improvements outside of VO_{2max} might be appropriate; there are various methods of achieving this, including resistance and plyometric training (Beattie et al., 2004). Knowledge of predicted training responsiveness can also lead to more personalised manipulation of common training factors such as volume, intensity, frequency and duration to improve exercise adaptation. As an example, it has been previously reported that the number of low responders to an aerobic training intervention could be significantly reduced, and even eliminated, with an increase in exercise intensity (Ross et al., 2015). Similarly, a recent paper found that an increase in exercise frequency and volume, with the same intensity, completely eliminated the occurrence of non-response to aerobic training (Montero and Lundby, 2017). The demonstrated predictive validity of this genetic algorithm potentially adds useful information to coaches, aiding in the interpretation of fitness assessments, and ensuring information is available for the planning of more effective training programmes.

The SNPs utilised in this study occur within genes demonstrated to affect either aerobic capacity, or the magnitude of improvements in aerobic fitness following exercise. Most of these SNPs occur in genes that affect the cardiopulmonary system or mitochondrial biogenesis. *VEGF* encodes for vascular endothelial growth factor, which influences the growth of new blood vessels in and around skeletal muscle. The C allele of this common polymorphism (rs2010963) increases expression of this gene, likely leading to increased blood vessel growth and hence greater oxygen availability during exercise (Ahmetov et al., 2008). *ADRB2*, which has two common polymorphisms (rs1042713 and rs1042714) included in this algorithm, encodes for the β_2-adrenergic receptor. This receptor is the site to which catecholamines bind, increasing cardiovascular parameters such as stroke volume and cardiac output. These two common polymorphisms are associated with increases in receptor density, leading to increased stroke volume, cardiac output, vasodilation, and bronchodilation, all of which increase oxygen delivery. These polymorphisms may also increase exercise-based lipolysis, improving performance at lower exercise intensities (Sarpeshkar and Bentley, 2010), and have previously been associated with both elite athlete status (Wolfarth et al., 2007) and maximal oxygen consumption (Moore et al., 2001). The *CRP* rs1205 polymorphism can lead to an increase in C-reactive protein release at both rest and during exercise, potentially negatively impacting VO_{2max} (Kuo et al., 2007b). *PPARGC1A* encodes for PGC-1α, the master regulator of mitochondrial biogenesis. G allele carriers at rs8192678 typically have higher VO_{2max} values following exercise training (Ring-Dimitriou et al., 2004). The SNPs used in this algorithm are not exhaustive, but represent those that have, to date, been well replicated. As other SNPs which modify improvements in aerobic fitness are discovered and replicated in multiple cohorts, their addition to this genetic algorithm would likely enhance its association with aerobic fitness improvements.

Regarding the practical application of these findings, astute coaches have long been aware that improvements in aerobic fitness following training vary extensively between athletes. This is true even when those athletes have similar training histories, dietary habits and lifestyles. In addition, prediction of adaptation to aerobic training is currently not possible using conventional physiological assessment tools (Timmons et al., 2010). This study suggests that a simple, non-invasive genetic test is associated with the magnitude of improvements in aerobic fitness following a training programme, and so may potentially help in the programming of training. The identification of athletes who are more likely to see smaller improvements allows for such athletes to follow a different training intervention, potentially with greater intensity (Ross et al., 2015), frequency (Montero and Lundby, 2017) or perhaps with an increased emphasis on repeated sprint or resistance training. This contrasts with the current best practice, which is the application of training to an athlete, and the measuring of that response. If the response is less than expected, then either the athlete is considered to have reached their potential, or a different training method is utilised. This trial and error approach is costly in terms of time. Given that a high-level sporting career can last around 10 years, a training cycle spent following ineffective training can seriously harm the athlete's performance. The ability to more accurately predict the magnitude of exercise response could potentially:

1. Improve training prescription accuracy, and therefore training efficiency
2. Enhance the personalisation of athlete-specific training programmes
3. Reduce the costly trial and error process of executing unnecessary and/or inefficient training modalities.

These results potentially represent an early step on the journey to a higher level of personalisation within the training process. A possible limitation of this initial study is the modest sample size (n = 42). Nevertheless, whilst modest, this sample size is similar to other research in this field (Del Coso et al., 2017a; Erskine et al., 2013; Santiago et al., 2010). This sample size is also representative of the size of a typical soccer squad (first and reserve teams), giving it real-world validity. The participants were all male, so it is not clear if the results would be applicable for females. In addition, the number of participants in the "low" group was small (n = 6); pre-test power calculations were not possible because the genetic results of the athletes were not available until completion of the study. With information regarding frequency of athletes expected to be in the "low" group now available, this information can be used to ensure adequate sample sizes in future. Further research should build on these initial findings in a larger cohort, other sports, and females, as well as studying interventions aimed at enhancing aerobic training response. The Yo-Yo IR1 test used in this study is a maximal test, and so scores are potentially influenced by participant motivation. Whilst none of the SNPs used in this study have been found to influence participant motivation, there is a small possibility that variation in these genes could influence exercise tolerance, and hence test performance (Pickering & Kiely 2017b). Additionally, improvements in Yo-Yo test performance may occur outside of adaptations in aerobic capacity, such as improvements in technical performance and anaerobic capacity. Future studies may wish to use laboratory-based tests to directly explore aerobic fitness improvements. Additionally, as no comparator arm was present, there is the potential that random-within subject variation contributed to the observed inter-individual variation (Atkinson & Batterham, 2015). Furthermore, the relative work or training loads of the small-sided games were not quantified via external methods such as through the use of GPS and/or accelerometers to determine distance travelled and running intensity, or via additional internal measures such as heart rate. Although training intensity was prescribed via RPE, other means of quantification may have proved useful. Additionally, controlling for a number of covariates, including player age, maturation, playing position, and training history/experience, outside of baseline testing data—in which there were no differences between groups—would have strengthened the conclusions of the present study. A final limitation is that there were no set progressions built into the small-sided games training program in terms of increasing the relative intensity of the exercise bouts in a periodised or linear manner, although variation was provided through changes in team and pitch size.

Finally, whilst the results of this study indicate that the current five-SNP algorithm has utility, the addition of a greater number of polymorphisms would likely enable it to become more precise. Indeed, it is envisioned that the current algorithm is not a definitive end-point, but instead an initial attempt to predict training response that will become more refined and precise as more information is available. Nevertheless, the fact remains that very little research has been done in utilising genetic information in sporting practice, despite there being an undoubted genetic influence on the magnitude of

adaptation following aerobic training. The novel findings of this study, even at this early stage in the evolution of such technology, should contribute to the further development of this area.

5. Conclusion

The results of this study indicate there is considerable inter-subject variability in response to aerobic training in a group of well-trained male soccer players. In addition, it also demonstrates that the magnitude of these improvements is associated with a genetic test comprised of five SNPs. This previously unavailable information has the potential to provide insight to coaches, medical practitioners, personal trainers and athletes, enabling more informed decision making and evidence-led customisation of training programmes aimed at improving aerobic fitness. This potentially aids athletes, and their support staff, in selecting the optimal training modality, allowing for a more personalised training approach, and, in future, the maximisation of training adaptations for all athletes.

CHAPTER 11: A GENETIC-BASED ALGORTIHM FOR RECOVERY

Chapter preface:

Post-exercise recovery is an important component of the adaptive process in athletes (Bishop et al., 2008). There is, however, an apparent trade-off between too much recovery, and hence too little stimulus, and not enough recovery, a balancing act which, if inexpertly negotiated, can drive issues such as underperformance, injury, and accumulation of residual fatigue (Mair et al., 1996; Soligard et al., 2016). As a result, considerable time and money is spent at the highest level in optimising athlete recovery processes, often with mixed results. This chapter outlines a study which utilised a commercially available Total Genotype Score to determine whether the magnitude of reduction in Countermovement Jump, a valid and reliable test of neuromuscular fatigue (Cormack et al., 2008a), following a repeated sprints session were associated with genotype score.

1. Introduction

Exercise training produces a variety of acute physiological challenges to the body, perturbing homeostasis and inducing a stress response, the overcoming of which leads to exercise adaptation. Successful adaptation is comprised of the accumulation of periods of stress-induced response (training), and periods of recovery, which takes place away from exercise (Bishop et al., 2008). This relationship is finely balanced, and if there is insufficient time between training sessions for recovery to occur, the athlete increases their risk of undue accumulation of fatigue, potentially resulting in acute underperformance, injury (Mair et al., 1996), illness (Schwellnus et al., 2016), and eventually non-functional overreaching and unexplained underperformance syndrome (UPS) (Soligard et al., 2016). These indicators of maladaptation are common in athletes, with 10-20% of endurance athletes suffering from UPS each year (Budgett, 2000). Overuse injury incidence is also frequent, with rates between 37% and 85% reported, depending on the sport (DiFiori et al., 2013; Wilber et al., 1995). The prevalence of these symptoms of stress-recovery imbalance at epidemic proportions indicates that there is a mismatch in knowledge of how to create stress and how to recover from it. Indeed, a search on PubMed yields over 37,000 papers with “exercise training” in the title and abstract. A similar result with “exercise recovery” as the search field only results in 13,000 papers.

At the cellular level, the physiological challenges induced by exercise include increased oxidative metabolism within the mitochondria, leading to increased formation of reactive oxygen species (ROS) (Fisher-Wellman & Bloomer, 2009). Under normal, non-exercise conditions, the body can neutralise ROS through its endogenous antioxidant defence system, which is comprised of enzymes such as superoxide dismutase (Belviranli & Gokbel, 2006). However, when ROS production is increased through exercise, an imbalance between ROS production and neutralisation occurs, leading to elevated oxidative stress (Fisher-Wellman & Bloomer, 2009). In turn, this elevates lipid peroxidation and tissue damage. Mechanical load also increases muscle damage (Baumert et al., 2016), initiating an

inflammatory response partially driven by pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNF) (Yamin et al., 2008).

These changes at the molecular and cellular level drive the whole-body symptoms of under-recovery that athletes and coaches are well aware of. Increases in plasma IL-6 levels occur following exercise (Robson-Ansley et al., 2007), and administration of exogenous IL-6 into athletes induces feelings of fatigue and impairs performance (Robson-Ansley et al., 2004). Increased IL-6 is also a risk factor for the development of UPS (Robson, 2003). Both TNF and IL-6 can act on the central nervous system (Ament & Verkerke, 2009), potentially decreasing the drive to exercise. Increased ROS and oxidative stress are also associated with a decrease in physical performance (Powers & Jackson, 2007), and increased feelings of muscle soreness following exercise (Konig et al., 2001).

A number of best practices for recovery have been put forward (Bishop et al., 2008; Leeder et al., 2012b; Soligard et al., 2016; Schwellnus et al., 2016). However, there is likely considerable inter-individual variation in the time course of exercise recovery (Nosaka et al., 1996). This is partially governed by genetic variation between individuals, with several single nucleotide polymorphisms (SNPs) already identified as potentially affecting the speed of post-exercise recovery (Baumert et al., 2016). Knowledge of this variation may enable athletes and support staff to create individualised recovery interventions based on the identification of individuals at increased risk of exercise induced muscle damage or oxidative stress (Del Coso et al., 2017a).

Whilst this emerging research of the impact of genotype on exercise recovery is interesting, the translation of these findings to the field are currently under-explored. Given the proposed impact of genetic polymorphisms on exercise recovery, the purpose of the present study was to attempt to bridge this gap, by determining whether a seven SNP algorithm successfully differentiated between the recovery speed of male soccer players. It is believed that individuals possessing a greater number of alleles associated with increased oxidative stress, muscle damage or inflammation would see a greater reduction in neuromuscular function post-training, and that this reduction would take longer to abate relative to those individuals in possession of a more favourable genetic profile.

2. Methods

2.1 Participants

18 male soccer players aged between 16-19 years of age from a college soccer academy volunteered to participate in this study. Each player had an average of 11 years' football training experience, and was actively competing in the English College Football Association Premier League.

2.2 Methodology

As part of their normal soccer training, and following 24-hours rest, the participants took part in a repeated sprint testing session. Both before, and immediately upon completion of this session, the participants underwent Countermovement Jump (CMJ) testing. This test was repeated at 24- and 48-hours post-training to monitor their recovery status. Participants were familiarised to all tests as they are regularly used during their normal soccer training. Within the CMJ trials, participants undertook three trials at each time point, which were averaged to give a mean score. Participants had two minutes' recovery between trials. Prior to the initial exercise bout and subsequent testing, players carried out a standardised 15-minute warm up, consisting of pre-activation exercises and dynamic drills. The initial exercise bout was comprised of two sets of seven 25m sprints undertaken outdoors on a soccer pitch. The recovery period was 30 seconds passive recovery between sprint reps, and 5 minutes passive recovery between sets. Previous work has demonstrated that similar high-intensity repeated sprint efforts induce neuromuscular fatigue—as evidenced by a reduction in CMJ height—for up to 72 hours post-exercise (Keane et al., 2015), along with elevations in Creatine Kinase (CK), increased sensations of muscular soreness (Keane et al., 2015), and general fatigue (Goodall et al., 2015). As such, this protocol is likely suitable as a method of examining neuromuscular fatigue and exercise-induced muscle damage in sporting contexts (Howatson & Milak, 2009).

The CMJ was chosen as it has previously been shown to be a reliable and valid measure of neuromuscular fatigue (Cormack et al., 2008a, 2008b, 2008c; McLean et al., 2010; Gathercole et al., 2015) that is widely used in sporting settings (Taylor et al., 2012). The CMJ was measured using Optojump (Microgate, Italy), a valid and reliable tool for the assessment of vertical jump height in the field (Glatthorn et al., 2011). Prior to undertaking each CMJ, participants were instructed to keep their hands on their hips throughout the jump to eliminate any influence of arm swing. If the arms lost contact with the hips, the jump was classed as a no-jump, and an additional jump was performed following two-minutes recovery. In each CMJ trial, participants began standing upright, then performed a fast downwards eccentric action followed immediately by a jump for maximal height. Individual results were expressed as height jumped in centimetres (cm). Average CMJ height was utilised, primarily as a recent meta-analysis reported it to be more sensitive than peak individual CMJ height when monitoring neuromuscular fatigue (Claudino et al., 2017); the authors of that meta-analysis therefore recommended average CMJ height as a method of assessing neuromuscular fatigue.

2.3 Genetic testing

Alongside the training programme, participants underwent genetic testing by DNAFit Life Sciences; this occurred via a sterile buccal swab. The samples were sent to IDna Genetics Laboratory (Norwich, UK), where DNA was extracted and purified using the Isohelix Buccalyse DNA extraction kit BEK-50 (Kent, UK), and amplified through PCR on an ABI7900 real-time thermocycler (Applied Biosystem, Waltham, USA). Through this process, genetic information regarding SNPs believed to affect post-exercise recovery speed (*CRP* rs1205, *GSTM1* & *GSTT1* INDEL, *IL-6* -G174C rs1800795, *IL-6R* rs2228145, *SOD2* rs4880, *TNF* G-308A rs1800629) was determined. The DNAFit test uses an algorithm to stratify participants into "slow", "medium" or "fast" recovery speed by utilising a Total Genotype Score (TGS) method. Each allele is given a score of between 0 and 4 points depending on the expected magnitude of its impact on post-exercise recovery speed. The strength of the rating was based on the evidence from cumulative literature results averaged over time. The sum of these points was combined to give an overall score. This method is identical to Jones et al. (2016), and similar to the methods used in other studies utilising genetic algorithms (Ruiz et al., 2009; Meckel et al., 2014; Pickering et al., 2018). An overall score of 40% or less is classed as a "fast" genetic recovery speed. Scores of 41-60% are classed as a "medium" genetic recovery speed. A score of >60% is classed as a "slow" genetic recovery speed. The athletes were blinded to their genetic results until completion of the final testing.

2.4 Statistical analysis

As this is not a genetic association study, but an observational study into the effects of TGS on exercise recovery, gene-by-gene analysis was not carried out. Instead, data pertaining to exercise recovery was compared to individual athlete TGS group. Means and standard deviations were calculated for whole group and sub-groups for both pre- and post-training (0h, 24h and 48h) test scores. CMJ height at the three post-training time points was converted to a percentage of pre-training height. Given the small sample size, along with the fact that significance testing is sensitive to low sample sizes, and doesn't inform as to the magnitude (Buchheit 2016), effect sizes (Cohen's d) were instead calculated for between group differences at the three post-training time points. The thresholds used were 0.2 (trivial), 0.5 (small), 0.8 (moderate), >0.8 (large) (Cohen 1988). Data were analysed using Microsoft Excel 15.29 (Microsoft Corporation, Redmond, WA, USA). All data are reported as mean ± SD.

3. Results

Overall, 12 participants were classed as having a fast recovery speed, with 6 participants having a medium recovery speed. No participants were found to have a slow recovery speed; based on an analysis of 17,000 samples tested by DNAFit (Pickering, unpublished data – detailed in Chapter 4), approximately 6% of all individuals within a population would be expected to be in the slow group. Within this sample population, it would therefore be expected that one participant would be in the slow group; the lack of such an individual in the present study is therefore not unusual. Table 10 shows the absolute CMJ results for the fast and medium genetic recovery speed groups. Figure 11 illustrates the

between group differences as a percentage over the 48-hour period following the exercise bout, along with effect sizes.

Group	CMJ Pre-Training	CMJ Post-Training	CMJ 24h Post-Training	CMJ 48h Post-Training
Fast (n=12)	37.6 ± 6.2 cm	37.0 ± 6.2 cm	36.0 ± 6.9 cm	37.0 ± 6.1 cm
Slow (n=6)	35.7 ± 5.6 cm	34.1 ± 6.3 cm	33.3 ± 5.5 cm	33.7 ± 5.6 cm

Table 10 – CMJ values for both groups across all time points.

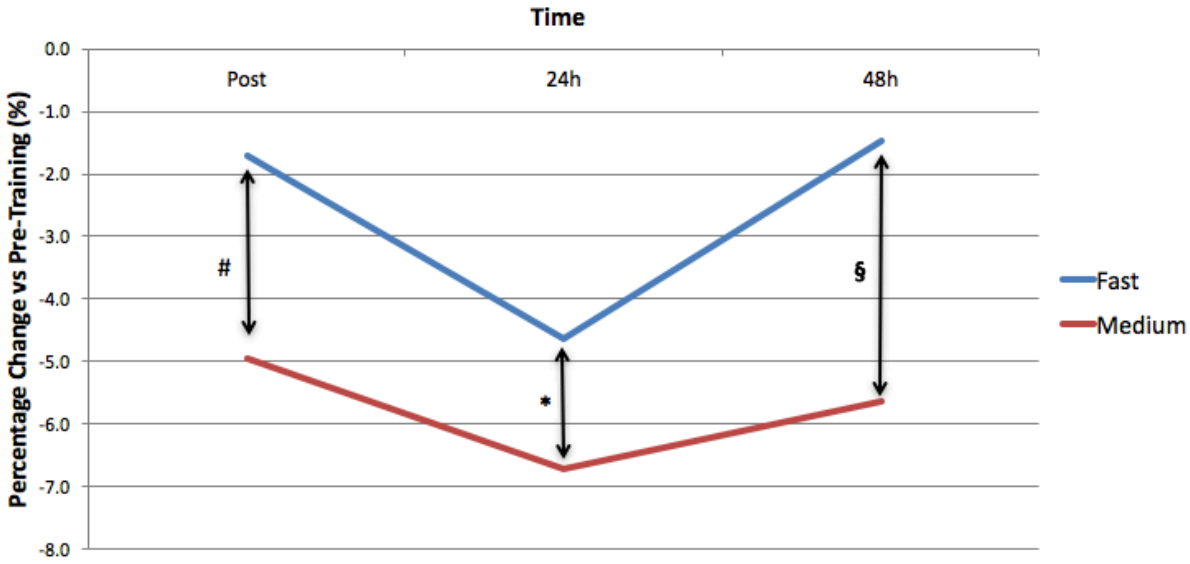

Figure 11 – Percentage change in CMJ height immediately post-training, 24h post-training, and 48h post-training as a percentage relative to pre-training values. Effect sizes are # = 0.7 (medium), * = 0.5 (medium), § = 1.0 (large).

4. Discussion

The results of this study indicate that rate of recovery, as measured by CMJ, is potentially modified by a total genotype score comprised of seven SNPs thought to impact exercise recovery. Overall, players in the "fast" genetic recovery speed group tended to demonstrate a smaller reduction in CMJ height relative to those players in the "medium" genetic recovery speed group, and were closer to baseline score following 48-hours of recovery. Immediately upon completion of the exercise bout, and 24 hours later, the magnitude of this effect was representative of a medium effect size. Forty-eight hours after training, this effect size had grown in magnitude to large. This suggests that these seven SNPs potentially modify recovery speed, such that individuals with more favourable alleles suffer a smaller percentage loss in CMJ height after repeated sprints, and have regained a greater percentage of their pre-training CMJ height 48 hours post-training. However, when interpreting these results, it is important to

consider that that the coefficient of variation in CMJ performance is ~3-5% (Gathercole et al., 2015), which, in the present cohort, equates to a ~1-1.6cm reduction in jump height following repeated sprint exercise. These values are similar to the reduction in jump height demonstrated here, so it is possible that these changes in performance may be due to both technical error and random within-subject variation. Furthermore, the reported baseline standard deviations suggest a smallest worthwhile change (0.2 x SD; Buchheit 2018) that corresponds to a value larger than the performance changes demonstrated here. As such, the real-world significance and utility of these results is unclear.

Nevertheless, the ability to predict the recovery time needed following intense exercise may be useful for several reasons. Firstly, it ensures that optimal recovery time can be given to individual athletes, reducing the fatigue that will accumulate across a training programme. This will ensure that the athlete is not placed at undue risk of suffering from injuries, of which the risk increases under fatigue (Dugan & Frontera, 2000), and may guard against the developing of unexplained underperformance syndrome. It may also be useful when planning the final physical conditioning session before a competition, with players genetically predisposed to slower recovery speeds having a longer rest period pre-competition. Finally, it may increase the motivation of individuals to carry out the correct recovery modalities post-training or post-competition, particularly if they are shown to have a slower recovery speed. However, the results from nutrigenetic research indicate that, at present, individuals do not always make behavioural changes based on genetic information (McBride et al., 2010); whether this is the case in highly motivated sports people is unclear.

The SNPs that comprise the genetic algorithm used here have previously been shown to potentially influence both the inflammatory response to exercise and the ability to tolerate oxidative stress. *IL-6* -G174C (rs1800795) has been shown to influence creatine kinase (CK) levels following eccentric exercise, with the C allele of *IL-6* associated with higher levels (Yamin et al., 2008; Lappalainen 2009). The *IL*-6 C allele is also associated with increased post-exercise plasma IL-6 (Huuskonen et al., 2009), which is associated with increased fatigue in athletes (Robson-Ansley et al., 2007). *IL-6R* (rs2228145) is also associated with post-exercise IL-6 levels, with the C allele associated with higher concentrations (Reich et al., 2007). Increases in plasma IL-6R concentrations are associated with increased C-Reactive Protein (CRP) levels post-exercise, as well as increased feelings of fatigue in athletes (Robson-Ansley et al., 2009). The *TNF* G-308A (rs1800629) polymorphism is associated with plasma CRP levels following aerobic exercise, with AA genotypes having higher CRP levels (Lakka et al., 2006). *CRP* (rs1205) alters plasma CRP concentrations, a common exercise recovery marker (Miles et al., 2007; Ingram et al., 2009), with G allele carriers having significantly higher CRP levels than AA genotypes (Eiriksdottir et al., 2009).

SOD2 (rs4880) encodes for manganese superoxide dismutase (MnSOD), which supports the dismutation of mitochondrial superoxide radicals into hydrogen peroxide and oxygen (Li et al., 2005). The T allele of this SNP is associated with increased CK post-exercise (Akimoto et al., 2010; Ahmetov et al., 2014b), although this relationship is complex and potentially modified by a subject's habitual antioxidant nutrient intake (Li et al., 2005). *GSTM1* (glutathione S-transferase M1) and *GSTT1* (glutathione S-transferase T1) are insertion/deletion polymorphisms, with deletion genotypes having poor

activity of the enzymes encoded for by these genes. Whilst these polymorphisms are well studied with regards to health and diet interactions (Palli et al., 2004), only one study has examined their impact on post-exercise muscle damage markers, with no significant differences between the inserted or deleted genotypes (Akimoto et al., 2010). These SNPs were included in the algorithm based on their theoretical impact on exercise recovery (Evelo et al., 1992; Vani et al., 1990).

The identification of athletes who may be genetically predisposed to increased recovery times can lead to the use of targeted recovery modalities, including nutritional interventions. Phillips and colleagues (2003) reported that 14 days of supplementation with vitamin E, omega-3 and flavonoids blunted the release of IL-6 and CRP following eccentric exercise. Similar results have been reported from other studies (Satoshi et al., 1989; Jouris et al., 2011; Sacheck et al., 2003). Other interventions that may enhance recovery include cold water immersion (CWI) and the use of compression garments, although the results are currently equivocal (Leeder et al., 2012b; Bleakley & Davison, 2009; Ascensao et al., 2010; Jakeman et al., 2009; Duffield et al., 2010; Duffield et al., 2008; Jakesment et al., 2010). It should be noted that exercise adaptation relies on the application of stress to the body, and the use of antioxidant supplements and CWI may blunt this adaptation (Draeger et al., 2014; Yamane et al., 2015), or in some cases reduce the speed of recovery (Close et al., 2006).

In addition to the potential lack of meaningful change in CMJ induced by fatigue within this study, there are some additional potential limitations. The use of CMJ as a measure of neuromuscular fatigue in football has recently been questioned (Carling et al., 2018), although it has been previously shown to be valid and reliable in this context (Cormack et al., 2008a, 2008b, 2008c; McLean et al., 2010; Gathercole et al., 2015). The cohort size is very modest (n=18). A standard soccer team is often comprised of 20-25 players; although at any given time some players may be injured and unable to train. The sample size in this study therefore represents a realistic size that coaches and practitioners may encounter in the real world. It is also representative of sample sizes often used in genetic pilot studies (Loy et al., 2015). However, this research requires replication in a larger cohort. In addition, this study had no participants with a TGS that suggested they had a slow genetic recovery speed, only medium and fast. The result of having a slow recovery speed is uncommon, with approximately only one participant for every sixteen tested expected to be in this category (Pickering, unpublished data), requiring very large sample sizes in order to recruit sufficient individuals into this category. The participants were all male, so it is unclear whether the results would be the same in females. Finally, it must be recognised that the use of this algorithm represents a crude measure, as many other SNPs doubtless affect exercise recovery. However, the results of this initial study are both novel and promising, such that further research in this field should assist in the development of personalised recovery guidelines.

5. Conclusion

This study demonstrates that there is variation in the rate of recovery from a repeated sprint exercise in a group of well-trained male soccer players, although the real-world meaningfulness of this variation is unclear (Carling et al., 2018). The use of a seven SNP genetic algorithm appears to potentially aid in the identification of those players who may require longer recovery times between intense exercise bouts, or who may most benefit from targeted recovery interventions. These findings are similar to those of Del Coso et al. (2017a; 2018a), although the SNPs utilised vary. The implications of these findings suggest that knowledge of genetic information may be important in individualising recovery timings and modalities in athletes; future research exploring differences in perception of fatigue and recovery, based on genetic variation, may also be warranted. Future research is also required to replicate these findings in a larger cohort, as well as in females, and attempt to demonstrate real world utility; nevertheless, the results potentially herald a further step towards an individualised training process.

CHAPTER 12 - CAN GENETIC TESTING PREDICT TALENT? A CASE STUDY OF FIVE ELITE ATHLETES

Chapter preface:

As explored in Chapter 8, there is increasing interest in being able to use genetic information to predict the likelihood of an individual becoming a future elite sports performer. Whilst, at present, the general consensus is that genetic information cannot (Webborn et al., 2015; Vlahovich et al., 2017a), and perhaps should not (Camporesi & McNamee, 2016) be used in this way, further exploration is required to determine whether it holds real-world potential. This chapter presents a study which utilises the most comprehensive panel of genetic variants associated with elite athlete status compiled within published literature. Using this expanded gene panel, Total Genotype Scores (TGS) were calculated for five elite athletes, including an Olympic Champion. These TGS were then compared to a reference population of 503 non-athlete controls, to determine the effectiveness of the TGS in discriminating athlete from non-athlete. This study has been submitted for publication, and is currently undergoing peer-review.

1. Introduction

Over the last thirty years, there has been a rapid increase in the appreciation of how genetics influences elite sports performance. General heritability studies have estimated the heritability of elite athlete status to be approximately 66% (De Moor et al., 2007), and an understanding of how specific genetic variants, such as *ACTN3* (Yang et al., 2003), may predispose towards elite performance has continued to grow. These advances have served to increase speculation that genetic testing may be used to identify individuals with an increased likelihood of achieving elite athlete status in the future, with some direct-to-consumer genetic testing companies offering this service to customers (Webborn et al., 2015).

However, at present, the scientific consensus is that genetic information is ineffective at identifying future talented performers (Webborn et al., 2015), and, furthermore, poses significant ethical problems (Camporesi & McNamee, 2016)—both aspects are discussed at length in Chapter 8. Previously, Williams & Folland (2008) incorporated 23 genetic variants associated with elite endurance performance in a data simulation, with subsequent results suggesting that there was only a 0.0005% chance of any single person in the world having the optimal form of all 23 performance-associated variants. A further issue is that, within this simulation, there was considerable similarity in polygenic profiles between individuals, with the clustered distribution of genotype scores limiting the emergence of genetic outliers, who might be predicted to be more likely to be elite athletes. Similar findings, relating to muscular strength and power characteristics, have also been demonstrated (Hughes et al., 2011). These issues have also been explored experimentally, most commonly via the use of Total Genotype Scores (TGS). Here, a score is assigned for each genotype of interest, and then summed into a final score for that athlete. For example, Ruiz and colleagues (2009) collected data on elite Spanish endurance athletes and controls. Whilst, on average, the athletes had a greater TGS for a panel of seven endurance-related polymorphisms

than non-athlete controls, there was considerable overlap in score between the populations, indicating that the predictive capability of this TGS was low. Indeed, it was determined that individuals with a TGS above 74.71% were over five times more likely to be elite athletes; however, only 43.5% of the elite athletes attained such a score. Similar results were reported for elite power athletes (Ruiz et al., 2010); again, the athletes had a higher average power TGS compared to controls and endurance athletes, but there was a large crossover of the standard deviations, indicating limited sensitivity and specificity.

Such evidence suggests that utilising a relatively low number of polymorphisms to identify elite athletes is unlikely to provide meaningful insights (Williams & Folland, 2008; Ruiz et al., 2009; Ruiz et al., 2010; Santiago et al., 2010). However, many more polymorphisms than the 23 or fewer utilised in the studies to date have been associated with elite performance. A recent literature review (Ahmetov et al., 2016) reported that at least 155 genetic markers have been associated with elite athlete status, with further associations recently emerging (Guilherme & Lancha Jr, 2017). Additionally, in a recent survey in the UK, 67% of athletes and 48% of support staff stated that genetic testing would form a valuable tool to talent identification processes within their sport (Varley et al., 2018a), suggesting that there is an appetite for genetic information within the sports performance world.

As such, further research in this area is clearly required. If genetic information is to offer utility in the identification of talented performers, it needs to be able to discriminate between elite performers and the general public. The aim of this investigation, accordingly, is to determine whether the use of an increased number of genetic variants as part of a TGS can achieve such a goal, through the utilisation of a case study approach with five elite athletes. It is believed that such a large scale TGS has not previously been utilised to potentially identify talented athletes, demonstrating the novelty of this case study.

2. Methods

2.1 Participants

The participants were five former or current high-level athletes. All participants gave written consent for their results and identity to be shared here. The study protocol was approved by the University of Central Lancashire Ethics Committee, in accordance with the Declaration of Helsinki.

Participant A (Andrew Steele) is a former 400m runner. He competed at one Olympic Games, winning a medal in the 4x400m relay. His personal best time is 44.94s.

Participant B (Greg Rutherford) is a former long jumper. He has competed at three Olympic Games, winning a Gold and a Bronze medal. His personal best distance is 8.51m.

Participant C (Craig Pickering) is a former sprinter. He competed at one Olympic Games, and has a World Championships Bronze medal in the 4x100m relay. His personal best 100m time is 10.14s. He also won a Silver Medal at the European Indoor Championships over 60m.

Participant D (Tom Lancashire) is a middle-distance runner, competing primarily over 1500m, the distance at which he was selected for an Olympic Games. His personal best 1500m time is 3:33:96.

Participant E (Andrew Lemoncello) is a long-distance runner, with a Marathon personal best time of 2:13:40. He competed at two World Championships, and one Olympic Games.

2.2 Genetic testing

Each participant volunteered a saliva sample, which was collected through sterile and self-administered buccal swabs. The samples were sent to AKESOgen, Inc (Peachtree Corners, GA, USA), where DNA was extracted from the saliva samples using Qiagen chemistry on an automated Kingfisher FLEX instrument (Thermo Fisher Scientific, Waltham, MA, US), following the manufacturer's recommended protocols and standard operating procedures. PicoGreen and Nanodrop measurements were taken to measure the quality and quantity of the DNA. Input to the custom testing array occurred at 200ng in 20μL. Amplification, fragmentation, and resuspension was performed using Biomek FXP following Affymetrix's high throughput protocol for Axiom 2.0. Hybridisation was performed for 24 hours at 48°C in a Binder oven, and staining and scanning of the arrays was performed using GeneTitan instrumentation (Thermo Fisher Scientific, Waltham, MA, US), all following the same Affymetrix high throughput Axiom 2.0 protocol. Data analysis was then performed using a raw CEL file data input into the Affymetrix Axiom Analysis Suite (Affymetrix, Santa Clara, CA, US).

2.3 Creation of Total Genotype Scores

In order to best examine the potential use of genetic information in identifying elite athletes, polymorphisms previously linked to elite speed-power and elite endurance athlete status were collated through a structured literature search.

Speed-power athlete status: 48 genetic variants associated with power athlete status were identified from two review articles (Ahmetov & Fedotovskaya, 2015; Ahmetov et al., 2016). Of these 48, one marker (*IL1RN*) could not be genotyped due to lack of coverage on the AKESOgen chip array. A further SNP, rs2854464 in *ACVR1B*, was added to the panel (Voisin et al., 2016). Three SNPs in the carnosine genes *CNDP1* and *CNDP2*, associated with elite power athlete status (Guilherme & Lancha Jr, 2017) were also not present on the chip array, and so were not assessed. Mitochondrial DNA (mtDNA) was not assessed. The effect allele of one SNP, rs11091046 in *AGTR2,* was reversed given the findings of a recent meta-analysis (Yvert et al., 2018).

Endurance athlete status: 68 genetic variants associated with endurance athlete status were identified from two review articles (Ahmetov & Fedotovskaya, 2015; Ahmetov et al., 2016). Of these, the genotype of 5 (*ADARA2A* 6.7/6,3kb*, BDKRB2* +9/-9*, COL5A1* rs71746744, *NOS3* 4A/4B*, PPP3R1* 5I/5D) could not be determined due to insufficient coverage. An additional SNP, rs10497520 in *TTN,* was added to the TGS (Stebbings et al., 2018). mtDNA was not assessed.

2.4 Scoring

For each genetic variant, a score of 0, 1 or 2 was given depending on the genotype of the athlete. A score of 2 represents the possession of two alleles associated with elite athlete status (e.g. CC for *ACTN3* rs1815739 within the power TGS); a score of 1 represents carriage of one such allele (e.g. CT for *ACTN3* rs1815739 within the power TGS); and a score of 0 represents the possession of no elite athlete-associated alleles for that genetic variant (e.g. TT for *ACTN3* rs1815739 within the power TGS). For each trait, the scores were then summated, divided by the total possible score, and multiplied by 100 to get a percentage. This method is identical to that employed in previously published research utilising a TGS to explore elite athlete status (Williams & Folland, 2008; Ruiz et al., 2009; Ruiz et al., 2010; Santiago et al., 2010). The analysis was performed in Excel 16.13.1 (Microsoft, Redmond, WA, USA).

2.5 Control population

In order to develop an adequate control population, genotype scores for 503 European Caucasians were downloaded from e!GRCh37 (http://grch37.ensembl.org/index.html) into a spreadsheet for analysis. For each genetic variant, a score of 0, 1, or 2 was given as per the speed-power and endurance TGS detailed previously. The sum of scores for each variant was then calculated, and converted into the TGS% as per the previously detailed method. Additionally, the mean and standard deviation score for this reference population were calculated.

3 Results

3.1 TGS Scores

Figure 12 shows the results of all five participants' speed-power TGS, as well as the mean score expected in European Caucasians. The three speed-power athletes (A-C) had the highest TGS, whilst the two endurance athletes (D & E) had the lowest. This trend held up in comparison to the mean score for European Caucasians, with the speed-power athletes having a higher than mean score, and the endurance athletes a lower than mean score.

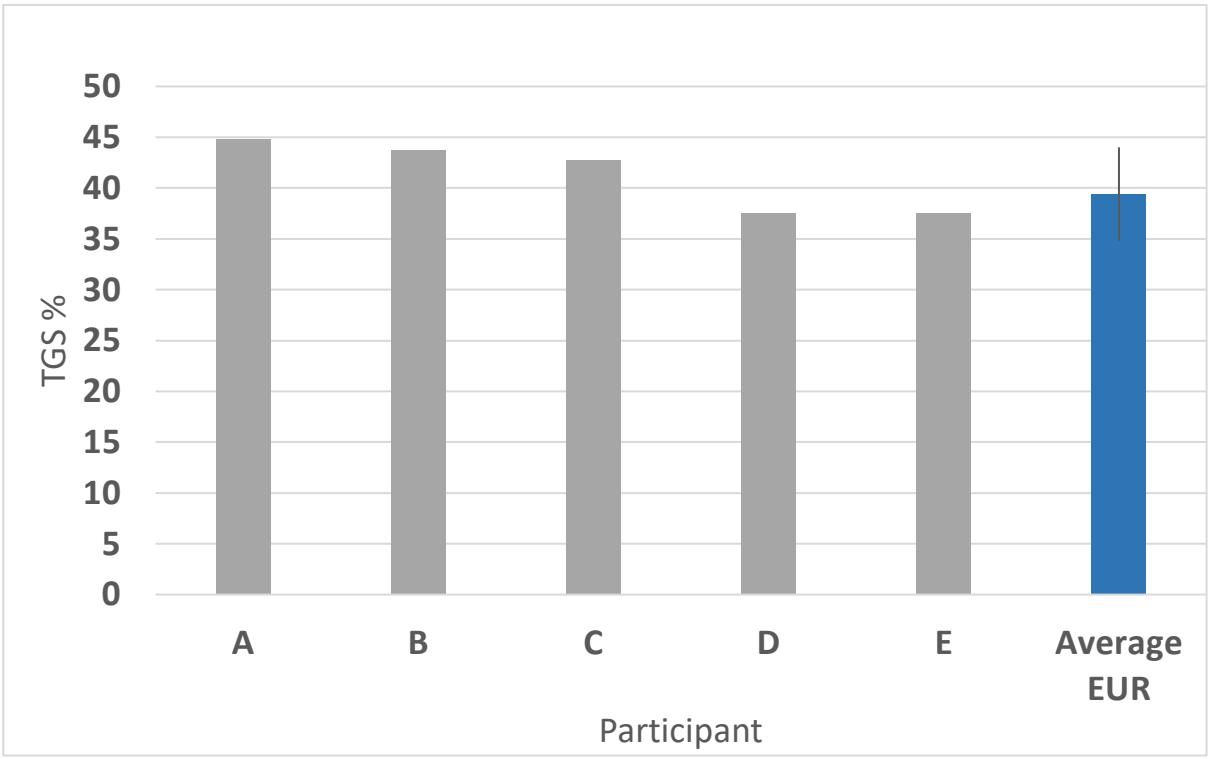

Figure 12 – The speed-power TGS of all participants, along with the mean (±SD) for European Caucasians.

In comparison, figure 13 demonstrates the results of the endurance TGS. Here, the two endurance athletes still have the lowest TGS—lower than the elite speed-power athletes and the mean for European Caucasians.

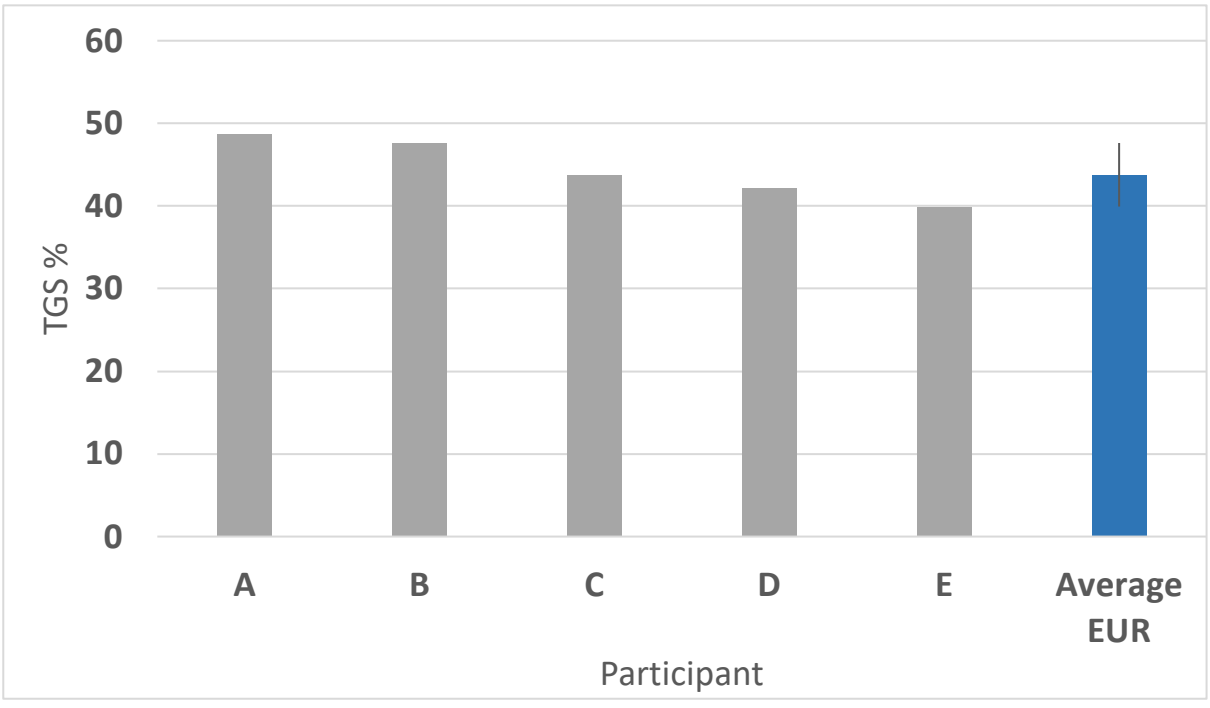

Figure 13 – The endurance TGS of all participants, along with the mean (±SD) for European Caucasians.

3.2 Comparison to previously published TGS

The next stage of the analysis involved calculation of the TGS from previously published research by Ruiz and colleagues (2009; 2010). The results for the speed-power TGS are shown in figure 14, and the results for the endurance TGS are in figure 15.

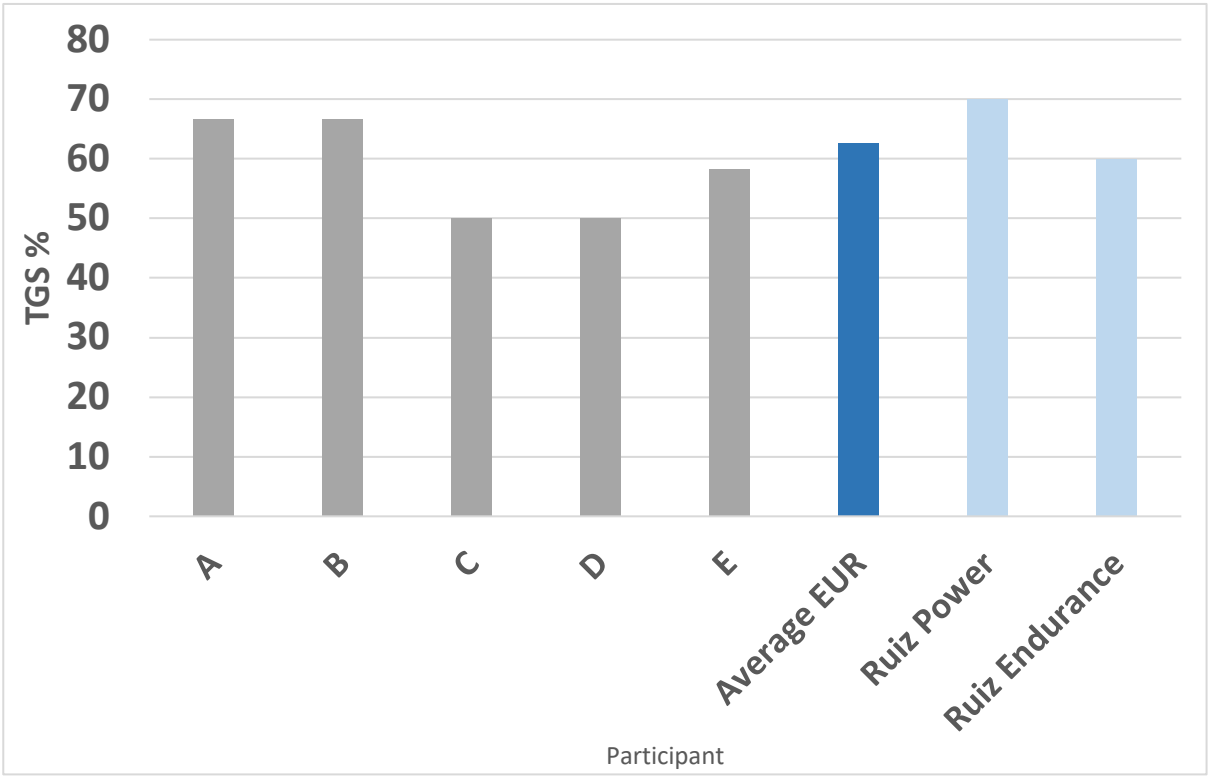

Figure 14 – The speed-power TGS from Ruiz et al. (2010) for all participants in the present cohort, the mean for European Caucasians, and elite power and endurance athletes from Ruiz's cohort.

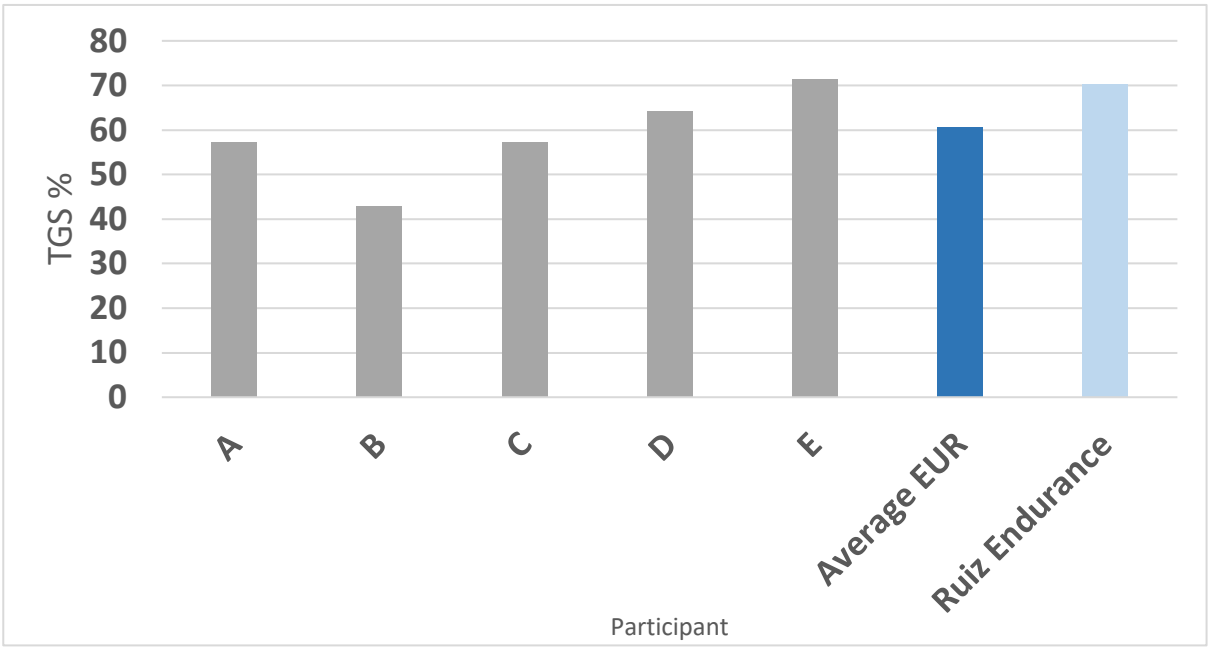

Figure 15 - The endurance TGS from Ruiz et al. (2009) for all participants in the present cohort, the mean for European Caucasians, and elite endurance athletes from Ruiz's cohort.

3.3 Non-athlete control results

The frequency distributions for 503 non-athletic Caucasian controls for both the power (figure 16) and endurance (figure 17) TGS were then calculated. In general, the results of the control population are fairly tightly distributed around the mean. Within the power TGS, no subject fell below a score of 26%, or above a score of 53%. Similarly, within the endurance TGS, no subject had a score below 34% or above 55%.

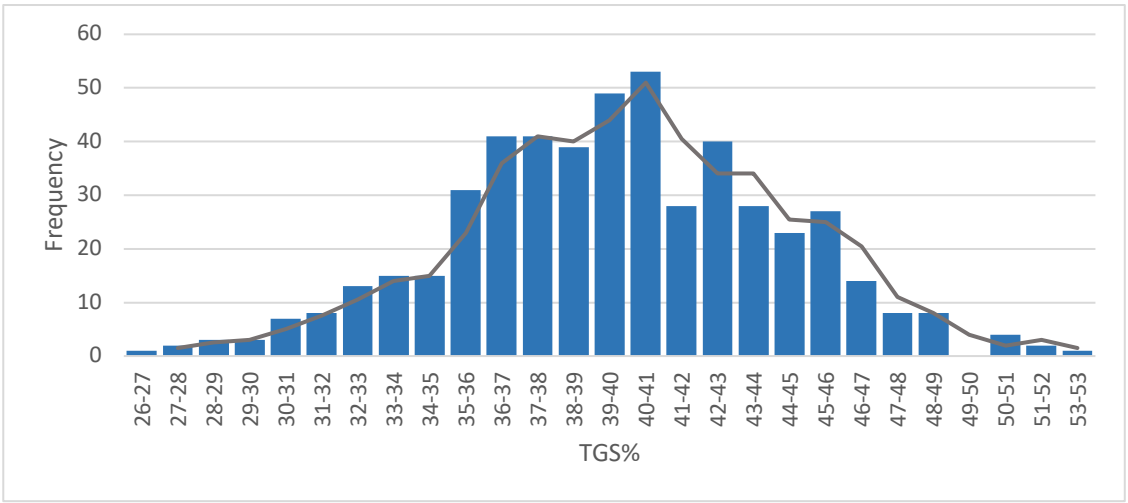

Figure 16 – Frequency distribution of power TGS% for non-athletic controls.

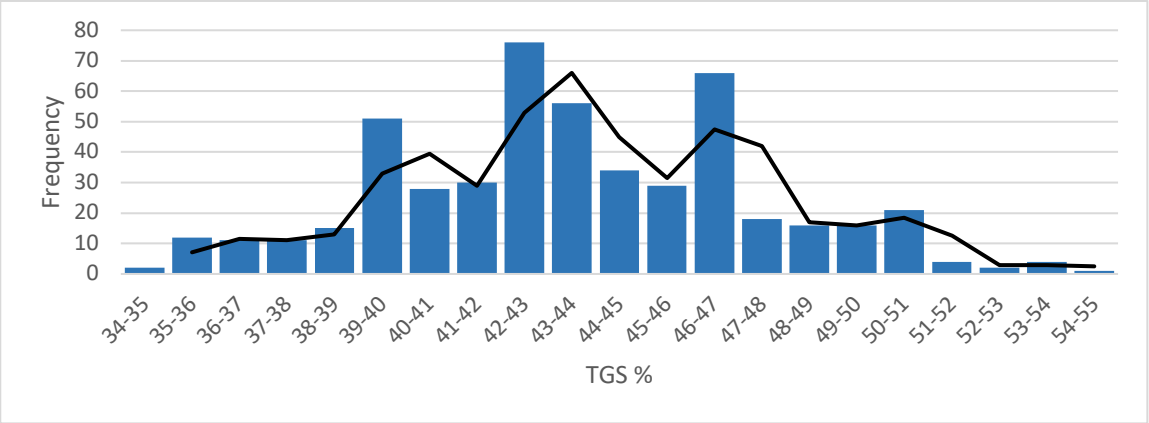

Figure 17 – Frequency distribution of endurance TGS% for non-athletic controls.

4 Discussion

Using a 48 SNP TGS of speed-power associated SNPs, there was a general trend for a higher score in the elite speed-power athletes (range 42.7-44.8%) compared to the elite endurance athletes (37.5%) in the present cohort. These findings also held up favorably compared to the mean score for Caucasian Europeans (39.4%); in this case, the speed-power athletes had a higher TGS than non-athlete controls, who in turn had a higher TGS than the elite endurance athletes. This suggests that the use of genetic information to identify talented performers may hold utility; however, both endurance athletes and two of the three power athletes were within one standard deviation of the non-athlete mean score. Indeed, in the 503 European reference samples utilised, 68 individuals had a higher speed-power TGS than athlete A, the highest scoring athlete in the present cohort. The highest score in the control population was a TGS of 50%, just over 2SDs greater than the mean.

The results for the 64 SNP endurance TGS further demonstrated the lack of utility of genetic testing for talent identification. Here, all three speed-power athletes (range – 43.8-48.7%) out-scored the endurance athletes (39.8 – 42.2%), who in turn scored lower than the mean for European Caucasians (43.8%). The SD for scores in the 503 European reference samples was 3.8%, with 82 control subjects having an endurance score >1SD outside of the mean. The highest score was 54.6%.

The comparisons to the previously published TGS utilised by Ruiz and colleagues (2009; 2010) provide some interesting results. In the present cohort, the elite endurance athletes scored more highly on Ruiz and colleagues' endurance TGS (64 and 71%) than the speed-power athletes. This is the opposite result to that seen when utilising the larger scale TGS developed for this study. This potentially suggests that the utilisation of fewer genetic variants within a TGS may enhance the predictive ability of such a model, potentially because the selected variants have a greater effect size. Larger sample sizes are required to further test this. Regarding the power TGS (Ruiz et al., 2010), the athletes in the present cohort all scored lower than the mean power score in the Ruiz cohort; two just outscored the mean for European Caucasians, whilst participant C—a European medalist over the 60m sprint—scored below the mean for European Caucasians, and was outscored by participant E, the long-distance runner. Again, this

is in contrast to the results of the present study, where the speed-power athletes all outscored the endurance athletes, suggesting that the larger scale TGS is potentially more sensitive in determining speed-power athlete status.

The two genetic variants with the most well-established associations with elite athlete status are *ACE* and *ACTN3* (Gayagay et al., 1998; Yang et al., 2003; Papadimitriou et al., 2016). Regarding *ACTN3*, the C allele of rs1815739 is consistently associated with elite speed-power athlete status, with two recent meta-analyses (Ma et al., 2013; Weyerstraß et al., 2018) finding that individuals with the TT genotype were significantly less likely to achieve elite speed-power athlete status compared to those with at least one C allele. The three speed-power athletes within the present cohort exhibit the full range of *ACTN3* genotypes (data not shown). Participant B, the highest achieving athlete of the cohort, has the CC genotype. Participant C, the short sprinter, possesses the CT genotype, whilst participant A, the Olympic 400m relay medallist, has the TT genotype. This latter result may be somewhat surprising given that this genotype is considered unfavourable for elite speed performance, a result which has also been demonstrated in 400m runners (Papadimitriou et al., 2016). The endurance athletes in this cohort possessed the CT and CC genotype respectively. Both of these genotypes would be considered slightly unfavourable for elite endurance athletes (Ma et al., 2013). This relationship, however, appears complex and poorly understood; whilst some studies suggest an association between the *ACTN3* T allele and elite endurance status (Yang et al., 2003), others do not (Papadimitriou et al., 2018).

The genotype results for *ACE* were similarly heterogenous (data not shown). For this genetic variant, the D allele is considered favourable for elite speed-power athlete status (Ma et al., 2013; Weyerstraß et al., 2018), with the I allele favourable for elite endurance athlete status (Ma et al., 2013). Within the speed-power athletes in this cohort, two athletes had the ID genotype, and one the II genotype; neither is considered optimal for elite sprint performance. Conversely, both endurance athletes had the favourable II genotype.

It's clear from the results of the non-athletic controls that there is a minimal spread of results within the general population. This similarity in polygenic profiles in non-athletes has previously been reported with a lower number of generic variants for both endurance (Williams & Folland, 2008) and strength/power (Hughes et al., 2011) phenotypes. Within this case study, none of the elite athletes were significant outliers in terms of TGS%, demonstrating that, for the polymorphisms tested, genetic information is not sufficient to discriminate between elite athletes and non-athletic controls

4.1 Would genetic testing have helped identify these athletes at a young age?

Based on the results presented here, it’s not clear that the use of genetic testing on these athletes during their teenage years would have identified them as potential future elite athletes relative to a group of non-athletes. It’s unlikely that this information would have proved more useful than traditional talent identification methods. Participant A, for example, was English Schools 400m Champion at age 16. Participant B is the British under-20 Long Jump record holder and former European under-20 Champion. Participant C won multiple national age group titles at under-15 and under-17, and the European under-20

Championships. Participant D won multiple junior national titles. Participant E also won national age-group championships. Consequently, given the failure of genetic information to provide insights over and above that provided by inspecting results and observing performances, the practical utility of such tests for the specific purpose of talent identification is not supported by these case study results.

4.2 Limitations

There are some limitations to the present study that must be considered when interpreting the results. Firstly, data on mitochondrial DNA (mtDNA) was not collected due to testing limitations. Mitochondrial haplotypes have been associated with elite athlete status, with different variations conferring an advantage or disadvantage in achieving elite athlete status for both speed-power and endurance athletes (Niemi & Majamaa, 2005; Castro et al., 2007; Scott et al., 2009; Eynon et al., 2011; Ahmetov et al., 2016). Furthermore, there were a small number of polymorphisms for which genotype data could not be collected due to a lack of coverage on the testing array. There is the potential that the athletes in this study may have held favorable versions of these variants, which would have increased their scores. However, even with these limitations, the TGS created for use in this study represents the most comprehensive gene score to appear in the published literature with regards to elite athlete status. Furthermore, the study utilised an unweighted TGS, with each variant having a score of 0, 1, or 2 depending on genotype. A weighted TGS, with genetic variants with demonstrably larger effect sizes receiving a greater score, may have proved more accurate. However, at present, very few genetic variants associated with elite athlete status have been adequately replicated, making the development of such a weighted TGS with a large number of variants difficult to achieve.

5 Conclusion

These results of this study suggest that, at present, the use of genetic testing to identify talented athletes appears to hold no clear predictive ability in discriminating between elite athletes and non-athletes. This is demonstrated in the current study by the TGS scores of five elite athletes, whose scores do not deviate substantially from mean population scores, nor do they reach the thresholds typically seen in elite athletes from other published TGS-elite athlete status associations (Ruiz et al., 2009; Ruiz et al., 2010), although the number of genetic variants used within these earlier studies was very small. Indeed, within this present cohort, and utilising the larger-scale TGS, all three of the elite power athletes had a higher endurance score than both the middle-distance and long-distance runners, demonstrating the lack of predictive power of the present TGS.

As a greater number of genetic variants associated with elite athlete status are identified, especially in areas involved in the psychological (Petito et al., 2016; Abe et al., 2018), anatomical (Marouli et al., 2017), and skill acquisition (Jacob et al., 2018) factors associated with elite athlete status, it is feasible that the predictive ability of future TGSs may improve; any improvement could further be aided by the use of weighted algorithms, where genetic variants with a relatively larger effect size achieve a higher relative score compared to variants with a smaller effect size. However, at present, and as clearly

illustrated by this case study involving elite athletes, the similarity of polygenic profiles within populations appears to limit the discriminatory power of genetic information to identify talented athletic performers.

Section 5 – Is there utility to genetic information in sport?

The content of this section draws on three previously published peer-reviewed papers. These papers are:

Pickering C, Kiely J. ACTN3, morbidity, and healthy aging. Front Genet. 2018;9:15.

Pickering C, Kiely J. Exercise Response Efficiency – A novel way to enhance population health? Lifestyle Genom. 2019.

Pickering C, Kiely J. The Development of a personalised training framework: Implementation of emerging technologies for performance. J Functional Morphol Kinesiol. 2019;4(2):25.

CHAPTER 13 – WIDER IMPLICATIONS: GENETIC INFORMATION FROM A PUBLIC HEALTH PERSPECTIVE

Chapter preface:

The sports science and medical worlds are inextricably linked, especially given the well-established and well-replicated preventative and treatment effects of exercise on a variety of diseases and health issues (Janssen et al., 2004; Frank et al., 2004; Latino-Martel et al., 2016; Pareja-Galeano et al., 2015); as a result, both worlds borrow ideas liberally from one another. Whilst this thesis has focused on the use and utility of genetic information within elite sport, there are potential wider applications emanating from these findings which may have important implications from a public health perspective. This chapter explores how the findings of the present thesis, as well as the wider body of exercise genetics research, might be used to improve public health. Part One contains a discussion on the modifying effects of *ACTN3* on morbidity and healthy aging, whilst Part Two explores the potential role genetic information can have on increasing both the effect of, and adherence to, an exercise training programme aimed at improving an individual's health. The first two parts have previously been published as review articles (Pickering & Kiely, 2018d; Pickering & Kiely, 2019b). Finally, Part Three, briefly explores how genetic information may be utilised to inform diet choice, with particular reference to the treatment and management of obesity and cardiometabolic health. Given the ever-increasing prevalence of obesity (Finucane et al., 2011), type-II diabetes, and other diseases associated with inactivity (Colditz 1999), a discussion focused on bridging the gap from elite sport to increased public health is warranted, and, hopefully, impactful, particularly given the increased healthcare burden these diseases create (Kelly et al., 2008; Wang et al., 2011).

PART ONE - *ACTN3*, MORBIDITY, AND HEALTHY AGING

1. Introduction

There is a frequently quoted axiom, often attributed to Benjamin Franklin, suggesting that "nothing is certain but death and taxes". Whilst recent scandals suggest that, for some, taxes may be optional, death remains a universal certainty. Fortunately, life expectancy has increased dramatically over a very short timeframe. Within the UK, for example, the expected lifespan has roughly doubled over the past 150 years, such that a child born today can expect to live until 80 years of age (Majeed 2013). Whilst reductions in infant mortality undoubtedly play a role, they only provide a partial explanation. This substantial leap in life expectancy is attributable to multiple—medical, societal, cultural, economic, and public health—factors. As a consequence, the number of people surviving into old age is rising, a trend which is expected to continue (He et al., 2016).

This trend has piqued interest in healthy aging, particularly as longer lifespans don't always correlate with sustained wellbeing (Christensen et al., 2009; Kuh et al., 2014). As health is multifactorial,

the research in this field has a wide scope, including disease avoidance and the maintenance of physical function into old age (Christensen et al., 2009; Kuh et al., 2014). Focusing on the latter, a number of physical performance measures are associated with healthy aging, including grip strength, standing balance, and walking speed, with lower scores in these tests typically associated with increased all-cause mortality (Rantanen, 2003; Cooper et al., 2010; Studenski et al., 2011). Accordingly, along with the absence of disease states such as type-II diabetes, the maintenance of muscle strength is an important component of healthy aging.

A second population to which muscle strength is important are elite athletes (Maughan et al., 1984; Hakkinen et al., 1989). With both muscle strength and elite athlete status being heritable traits (De Moor et al., 2007; Silventoinen et al., 2008), over the last twenty years there has been an increased focus on identifying the specific genes and single nucleotide polymorphisms (SNPs) affecting the inter-individual variation evident in athletic performance (Timmons 2011; Hughes et al., 2011). At present, over 100 SNPs associated with elite athlete status (Ahmetov & Fedotovskaya, 2015) and the exercise training response (Bray et al., 2009) have been identified. One SNP with a well-established influence on muscle phenotype is rs1815739, a C-to-T base substitution in *ACTN3* (Yang et al., 2003; Ma et al., 2013). This SNP results in the transformation of an arginine base (R) to a premature stop codon (X), with X allele homozygotes deficient in the α-actinin-3 protein (North et al., 1999). The main function of α-actinin-3 appears to be as a structural protein, forming part of the Z-line of the muscle fibre, which acts to anchor the actin filaments within the sarcomere (Yang et al., 2009). This protein is expressed exclusively in type-II muscle fibres, and as a result, XX genotypes tend to have a lower percentage of these fibres (Vincent et al., 2007). As such, the XX genotype tends to be significantly under-represented in elite speed, power, and strength athletes (Yang et al., 2003; Roth et al., 2008), although these results are not unequivocal (Sessa et al., 2011; Scott et al., 2010).

Both strength and muscle mass are protective against all-cause mortality in the elderly (Li et al., 2017). As *ACTN3* genotype can modify muscle phenotypes, this section will explore the relationship between this common polymorphism in *ACTN3* and healthful aging, with a particular focus on muscle. Such exploration provides a basis for an enhanced understanding of indivdualised risk factors for the morbidities associated with the aging muscle, and may soon guide the customisation of prophylactic exercise interventions such as resistance training.

2. *ACTN3*, muscle mass, and healthy aging

Sarcopenia is the loss of skeletal muscle mass and function associated with increased age (Rosenberg 1997; Cruz-Jentoft et al., 2010). This process begins relatively early in life, with reported onset at age 25 (Lexell et al., 1988), a 10% loss in peak lean mass at age 40, and 40% loss at age 70 (Porter et al., 1995). This loss of muscle mass and strength can be troubling for a variety of reasons, such as a reduction in overall physical function (Janssen et al., 2002; Rantanen 2003) and an increase in fall risk (Wickham et al., 1989). In knock-out (KO) mouse studies, those without *ACTN3* have a greater

muscle mass loss with aging (Seto et al., 2011b); the question arises - are these results mirrored in humans?

A number of studies have examined the impact of *ACTN3* on muscle strength and function in an elderly population. Delmonico and colleagues (2008) undertook an observational study of over 3000 well-functioning elderly participants over a five-year period. In males, increases in 400m walk time were significantly greater in XX homozygotes than RR genotypes, with a non-significant difference between XX homozygotes and RX genotypes ($p=0.075$). In females, RR genotypes had approximately a 35% lower risk of persistent lower extremity limitation (defined as difficultly walking 400m or climbing 10 steps without resting) than XX genotypes. Interestingly, there were no significant differences between genotypes with regards to other muscle and performance phenotypes. Kikuchi and colleagues (2015) reported a similar loss of function in elderly Japanese individuals, with a significantly poorer chair stand test score in XX genotypes compared to RR and RX genotypes. Judson et al. (2011) examined *ACTN3* genotype interaction on fall risk in over 4000 elderly Caucasian females. Here, individuals with at least one X allele had a significantly increased risk of falling compared to R allele carriers; this was true at both baseline and at multiple follow-up points. These results were mirrored by Frattini et al. (2016), who reported that falls were more prevalent in XX genotypes than R allele carriers. Walsh and colleagues (2008) reported that, in females, the XX genotype was associated with significantly lower total-body and lower-limb fat free mass (FFM). In addition, these female participants had lower peak torque values compared to R allele carriers. There were no genotype effects in male participants. Similar lower values for muscle mass in elderly female XX homozygotes were reported by Zempo et al. (2010), with mean thigh cross-sectional area 4.5cm^2 lower in XX vs R allele carriers ($p<0.05$). Finally, Cho and colleagues (2017) reported a significantly higher sarcopenia risk in XX genotypes than RR genotypes in a cohort of elderly Koreans. However, other studies have found no effect of this polymorphism on muscle phenotype and function in the elderly (San Juan et al., 2006; Bustamante-Ara et al., 2010; McCauley et al., 2010), and one study (Lima et al., 2011) reported significantly greater FFM values in X allele carriers.

The general consensus from these studies is that *ACTN3* genotype exhibits a potentially modifying effect on muscle mass, maintenance of muscle function, and sarcopenia risk in elderly individuals, with the R allele associated with greater maintenance of strength and physical function, along with increased sarcopenia protection. From a muscle phenotype perspective, an association between *ACTN3* genotype and sarcopenia seems logical; specific type-II muscle fibre atrophy is a hallmark of sarcopenia (Lexell et al., 1988; Fielding et al., 2011), and, in athletic populations at least, the R allele is associated with an increased proportion of type-II muscle fibres (Vincent et al., 2007). This ability to more effectively maintain fast-twitch fibre size and mass with age is perhaps the mechanism by which *ACTN3* genotype modifies the age-related loss in muscle function, and concurrent fall and sarcopenia risk.

Given that resistance training is an important tool in sarcopenia prevention and treatment (Roth et al., 2000), and that *ACTN3* genotype may modify resistance training adaptations (Kikuchi & Nakazato, 2015), it is important to explore whether such a relationship exists in an elderly population. In elderly Caucasian females undertaking a 12-week resistance training programme, Pereira and colleagues (2013)

reported that *ACTN3* RR genotypes exhibited greater leg extension and bench press one-repetition maximum (1RM) improvements than XX genotypes. Delmonico et al. (2007) put elderly participants through a 10-week unilateral knee extensor strength training programme. In the male sub-group, absolute peak power increased to a greater extent in RR homozygotes compared to XX homozygotes, although this difference was not significant (p=0.07). In females, relative peak power change was greater in the RR group compared to the XX group. At present, these are the only two studies examining the impact of *ACTN3* on resistance training response in an elderly cohort, with the consensus being that the R allele, and specifically the RR genotype, is associated with enhanced strength and power improvements. Based on these findings, it appears that elderly *ACTN3* R allele carriers are more responsive to resistance training.

3. *ACTN3* genotype and bone mineral density with aging

Alongside age-related loss of muscle mass and function, a further risk factor is the loss of bone mineral density (BMD) and its related disease state, osteoporosis, with a well-established association between lower BMD scores and increased all-cause mortality (Browner et al., 1991; Johansson et al., 1998), stroke death (Browner et al., 1991), and fracture risk (Marshall et al., 1996). A small number of studies have examined the interaction between *ACTN3* genotype and BMD loss in elderly populations. Min et al. (2016), for example, reported a significant difference in BMD at both the spine and pelvis between genotypes, with XX and RX genotypes having lower scores than RR genotypes. Cho and colleagues (2017) reported similar findings, although the lower BMD in XX genotypes wasn't significant after covariate correction (p=0.075). Yang et al. (2011) found that, in postmenopausal women, *ACTN3* genotype was significantly associated with BMD, with XX genotypes having the lowest scores. Accordingly, overall it appears that the *ACTN3* R allele is somewhat protective against age-related BMD loss.

As discussed above, *ACTN3* genotype is likely associated with muscle function in the elderly. This may be the driving force between genotype differences in BMD, with individuals possessing greater muscle function able to be more active day-to-day. Such individuals are subsequently more likely to experience regular skeletal loading, thereby promoting structural maintenance, and diminishing BMD loss over time. Indeed, grip strength is positively correlated with BMD (Iida et al., 2012), as is increased muscle mass (Visser et al., 1998), indicating that perhaps the increased muscle mass and strength associated with the R allele is protective in this manner. However, using KO mice, Yang and colleagues (2011) reported a lower BMD in mice deficient in α-actinin-3. They reported evidence that α-actinin-3 is expressed in bone tissue and involved in osteogenesis, with KO mice having a reduced osteoblast and increase osteoclast activity. Perhaps both mechanisms play a role in the relationship between *ACTN3* and BMD, with further research required to understand the relative contributions of each.

4. *ACTN3* genotype and metabolic health with aging

Alongside muscle and BMD loss, aging populations also have to contend with an increased prevalence of a number of metabolic issues, including insulin resistance and type-II diabetes (Gunasekaran & Gannon, 2011; Suastika et al., 2012). These disease states are associated with a reduced mortality (Panzram, 1987), as well as an increased risk of further health issues (Williams et al., 2002) and cognitive decline (Strachan et al., 1997). Given that higher levels of muscle mass are associated with better insulin sensitivity (Srikanthan & Karlamangla, 2011), and that *ACTN3* genotype can modify muscle cross sectional area and fibre type, there is the potential that *ACTN3* genotype may alter type-II diabetes risk, either directly or indirectly. There is a paucity of research in this area; however, Riedl et al. (2015) reported that the prevalence of XX genotypes was greater in type-II diabetes patients than controls, indicating that the X allele may increase risk, although there were no differences between genotypes in terms of metabolic control or obesity. Research on *ACTN3* KO mice indicates that deficiency of Actn3, characterised by the XX genotype, does alter skeletal muscle metabolism (MacArthur et al., 2007), potentially by increasing fatty acid oxidation and glycogen storage.

As of yet, any relationship between this SNP and type-II diabetes requires further elucidation. The tentative findings of Riedl and colleagues (2015) are further complicated by research on the relationship between *ACTN3* and extreme longevity. In a cohort of Spanish centenarians, the XX genotype frequency was the highest reported in non-athletic Caucasians (24%), although there were no significant differences between X allele frequency in centenarians and controls (Fiuza-Luces et al., 2011). The authors concluded that this preliminary data suggests a potential survival advantage of the XX genotype. Similar complex results were found in a cohort of Japanese centenarians. Whilst there were no significant differences in genotype distribution between centenarians and controls, the frequency of the XX genotype in supercentenarians (over 110 years) was the highest seen in a non-American population, at 33% (Fuku et al., 2016). Indeed, whilst it appears that the evidence suggests that the R allele may confer a longevity advantage, likely mediated through its impact on muscle function, bone health, and metabolic wellbeing as discussed in this section, the lack of increased RR genotype frequencies seen in centenarians (Fiuza-Luces et al., 2011; Fuku et al., 2016) does not support this. Such a finding is mirrored in the longevity of elite athletes, with elite endurance athletes tending to live for longer than power athletes (Sarna et al., 1993; Teramoto & Bungum, 2009; Clarke et al., 2015). As the R allele is more prevalent in elite power athletes than elite endurance athletes (Yang et al., 2003), this again appears to suggest a paradox. The mechanisms underpinning the longevity advantage of elite endurance athletes is currently unclear, although there is the potential that the enhanced cardiorespiratory fitness exhibited by elite endurance athletes offers greater longevity than the improved muscle strength and function expected in former elite power athletes (Wisloff et al., 2005). This is particularly pertinent given evidence of more efficient aerobic metabolism in XX homozygotes (North 2008). Alternatively, the X allele could confer some as of yet unclear survival benefit; if this is the case, then there is the possibility that RX heterozygotes may have the greatest longevity benefit, by enjoying the benefits associated with each allele. Such an explanation would provide a potential mechanism explaining the lack of expected increases in RR genotypes in centenarian populations.

Nevertheless, given that loss of muscle mass increases risk of insulin resistance—a precursor to type-II diabetes (Srikanthan & Karlamangla, 2011)—and that type-II diabetes itself increases the risk of sarcopenia (Park et al., 2009; Kim et al., 2010), it appears that *ACTN3* genotype may modify type-II diabetes risk in the elderly. Again, it would be expected that the R allele, which is associated with increased muscle mass and performance, would be protective against age-related metabolic decline. Further research in this field should attempt to uncover such a relationship, should one exist.

In addition, *ACTN3* may alter health through other metabolic disturbances. In mouse models, there is evidence that the XX genotype may be protective against obesity (Houweling et al., 2017), although as of yet this association has not been replicated in humans (Moran et al., 2007; Houweling et al., 2017), with Deschamps and colleagues (2015) reporting increased obesity in XX genotypes. Similarly, there is evidence in younger populations that this polymorphism may affect other health markers, such as blood pressure (Deschamps et al., 2015) and high-density lipoprotein cholesterol (Nirengi et al., 2016); in both cases, the X allele was beneficial, although it is not clear if this is clinically meaningful, with further replication required.

5. Is this trifecta caused by *ACTN3*'s influence on muscle?

So far, this chapter has discussed the potential influence of *ACTN3* on three conditions associated with poorer outcomes with aging; sarcopenia and the resulting loss of muscle function, a loss of BMD, and a potential increase in metabolic disturbances, such as insulin resistance. These conditions likely have some degree of inter-relation; a loss of muscle function is likely associated with a lack of movement, which in turn reduces bone loading and turnover, leading to a loss of BMD (Vincent & Bray, 2002; Korpelainen et al., 2006). This loss of movement capacity could further cause a behaviorally-mediated reduction in type-II muscle fibres, further reducing muscle strength and function. Again, this loss of function might change habitual movement behaviors, thereby subsequently altering the metabolic profile of the individual and increasing the likelihood of some negative metabolic changes.

Accordingly, it seems feasible to speculate that the impact of *ACTN3* on these three risk-factors occurs either due its directly modifying effect on skeletal muscle, or through separate mechanisms for all three. This raises the question of whether elderly X allele carriers have lower BMD because they have less muscle mass and physical function, or if there is mechanism through which *ACTN3* influences bone turnover and mineral content. As detailed in section 3 above, there are tentative results that suggest *ACTN3* genotype influences both of these considerations, although whether its influence is greater on one than the other is currently unclear. As the results regarding *ACTN3* and insulin resistance are under-explored (Riedl et al., 2015), this leg of the trifecta is the most unknown; whilst there is a mechanism underpinning muscle mass and insulin resistance (Srikanthan & Karlamangla., 2011), and *ACTN3* does modify muscle mass and type in athletic cohorts (Vincent et al., 2007), it is not clear whether this holds true in the elderly.

If, as seems likely, the potentially modifying effect of *ACTN3* genotype on these three morbidities occurs primarily, although not exclusively, through its role in regulating muscle fibre type and strength, then this further underscores the need for elderly adults to undertake resistance training in order to maintain their health and function as they age. Whilst there is a clear protective effect of resistance training on the reduction of sarcopenia (Johnston et al., 2008), enhancing BMD (Rhodes et al., 2000), and reducing risk of insulin resistance and type-II diabetes (Dunstan et al., 2002) in the elderly, the insights outlined here do suggest some additional questions. Do those with the XX genotype, who would be expected to exhibit smaller improvements with resistance training, need to increase their training frequency and/or intensity (as suggested with regards to aerobic endurance training by Montero & Lundby, 2017), or should they undertake lower-load, higher-volume resistance training, as suggested by Kikuchi and Nakazato (2015) and supported by Jones et al., (2016)? Do other genetic variants, such as those found in *ACE* (Pescatello et a., 2006) or *AGT* (Aleksandra et al., 2016), influence the resistance training response in the elderly, and to what extent? There is also the possibility that *ACTN3* genotype may interact with these other genetic variants to modify the aging process in individuals. This has perhaps been most well studied in regard to *ACE* I/D, which is a polymorphism in the gene encoding for angiotensin-converting enzyme. Here, the results are equivocal, with some studies finding no effect of the *ACE* I/D polymorphism on muscle phenotype (McCauley et al., 2010; Garatachea et al., 2012), and others reporting that it modified the response to resistance training (Pereira et al., 2013), both on its own and in combination with *ACTN3.* Like *ACTN3, ACE* may also affect longevity through a variety of different pathways, including metabolic disease risk (Kajantie et al., 2004), blood pressure control (Yoshida et al., 2000; Santana et al., 2011), and Alzheimer's disease risk (Narain et al., 2000). Further work exploring the impact of resistance training on the elderly should perhaps take into consideration differences in genotype, either for single or multiple SNPs, to inform the design of more efficient and effective personalised exercise guidelines targeting positive outcomes for this population.

6. Conclusion

Variation in *ACTN3* has a demonstrable, clear and robust effect on muscle phenotypes in young, athletic populations (MacArthur & North, 2007; Vincent et al., 2007). Based on the research cited in this review, it appears to also have a modifying effect on muscle strength, size and function in the elderly (Walsh et al., 2008; Delmonico et al., 2008; Frattini et al., 2016), as summarised in figure 18 below. In particular, the R allele of *ACTN3* tends to be associated with better maintenance of muscle mass, strength and function (Delmonico et al., 2008), a greater adaptive response to training (Pereira et al., 2013), and is protective against the development of sarcopenia (Cho et al., 2017). There also appears to be a (less robust) relationship between *ACTN3* genotype and BMD in the elderly, with the R allele again being protective (Min et al., 2016; Cho et al., 2017). It is not clear whether this is due to *ACTN3* directly influencing bone metabolism, or whether the increased muscle mass and function of R allele carriers leads to greater bone loading, and therefore BMD maintenance. Similarly, there is an unclear relationship between *ACTN3* genotype and metabolic health; one study (Riedl et al., 2015) indicates that the XX genotype is present with an increased frequency in type-II diabetes patients, but clearly further research is required to better understand this relationship. Overall, whilst this indicates that the R allele should be

associated with increased health and function in the elderly, the picture is made more complex by research on centenarians (Fiuza-Luces et al., 2011; Fuku et al., 2016); in this case, the XX genotype is potentially more frequent in those over 100 years of age, although such a relationship is not statistically significant. If further research does support the early evidence that the *ACTN3* R allele is associated with a decrease in frailty risk factors, then knowledge of *ACTN3* genotype may better inform patients and medical practitioners as to each individuals' risk factors. This information could consequently inform personalised management strategies for the aging individual.

ACTN3		
	RR	**XX**
 Muscle function	• ↑ type-II fibre percentage • ↑ muscle function • ↓ fall risk • ↓ risk of sarcopenia	• ↓ muscle mass • ↓ muscle function • ↑ fall risk • ↑ risk of sarcopenia
 Bone Health	• ↑ BMD	• ↓ BMD
 Metabolic Health	• Potential ↓ in risk of developing insulin resistance • Unclear effect on obesity and blood pressure • Potentially ↓ HDL-C	• Potential ↑ in risk of developing insulin resistance • Unclear effect on obesity and blood pressure • Potentially ↑ HDL-C

Figure 18 – A summary of the impact of polymorphisms within *ACTN3* and healthy aging.

PART TWO - EXERCISE RESPONSE EFFICIENCY – A NOVEL WAY TO ENHANCE POPULATION HEALTH?

1. Introduction

Obesity, the condition of excess body fat or adipose tissue (Sweeting 2007), has become increasingly prevalent over the last thirty years (Finucane et al., 2011; Flegal et al., 2012). Between 1980 and 2008, mean Body Mass Index (BMI) increased globally by 0.4 kg/m^2, resulting in 1.47 billion adults being categorised as overweight (BMI ≥ 25 kg/m^2), and 503 million adults classified as obese (BMI ≥ 30 kg/m^2) (Finucane et al., 2011). These increases were most pronounced in Western countries, with the US—in which 35% of all adults are classed as obese—leading the way, closely followed by the UK and Australia (Finucane et al., 2011; Flegal et al., 2012). As obesity is recognised as a leading cause of a

number of co-morbidities, including cardiovascular disease, type-II diabetes, dyslipidemia, and cancer (Must et al., 1999; Gallus et al., 2014), these increased obesity rates represent a significant healthcare burden globally (Kelly et al., 2008; Wang et al., 2011), with the costs associated with treating obesity and its related diseases forecast to increase by up to $66 billion per year in the US and £2 billion per year in the UK by 2030 (Wang et al., 2011). As a result, considerable effort is being expended by public health bodies towards preventing and treating obesity (Must et al., 1999; Kelly et al., 2008).

However, so far, these efforts have done little to arrest the increasing rate of obesity. In part, this is due to the complex, multifactorial nature of obesity—whilst tempting to believe that obesity is merely a relative overconsumption of energy, the reasons underpinning this can be varied, and include increased sugar intake, increased portion sizes, alteration of gut microbiota, and genetic predispositions, along with societal and cultural influences (Friedman, 2009; Sahoo et al., 2015). However, a commonly cited reason for the recent explosion in obesity rates is that of a lack of physical activity (Janssen et al., 2004; Frank et al., 2004). In the US, the increase in obesity rates occurred alongside a significant reduction in leisure time physical activity, with no change in caloric intake (Ladabaum et al., 2014), suggesting that a lack of physical activity is potentially a major driver of the increase in obesity rates, at least in the US, where just under 50% of adults report no leisure time physical activity (Ladabaum et al., 2014). Furthermore, increasing physical activity has been shown to promote fat loss (Ballor & Keesey 1991; Boutcher 2010; Hazell et al., 2014), suggesting that physical activity could be important in the prevention and treatment of obesity and its related co-morbidities.

Alongside the associations between a lack of physical activity and obesity, and increased physical activity and weight loss, physical activity also reduces the risk of a number of other chronic diseases, including cancer (Latino-Martel et al., 2016) and cardiovascular disease (Myers et al., 2015), and has demonstrated efficacy as a treatment for type-II diabetes (Grace et al., 2017). As a result, physical exercise has been termed a “polypill” (Piepoli 2005; Fiuza-Luces et al., 2013; Pareja-Galeano et al., 2015; Sanchis-Gomar et al., 2015), with wide-ranging health benefits; indeed, the positive health benefits of exercise can be greater than a comparative treatment with drugs, particularly with regards to cardiovascular disease (Fiuza-Luces et al., 2013; Sanchis-Gomar et al., 2015).

Accordingly, it’s clear that physical activity and exercise have important, wide-ranging health promoting aspects, serving to both reduce the risk of chronic disease and obesity (Janssen et al., 2004; Frank et al., 2004), and serve as a treatment to these issues (Epstein & Goldfield, 1999); as a result, exercise can be thought of as medicine (Sallis 2009). However, current rates of physical activity in adults are low, having declined over the past thirty years (Ladabaum et al., 2014) in correlation with a large increase in obesity and other chronic disease rates. As such, there a plausible relationship between the demonstrated reduction in physical activity, and the increase in obesity seen globally. Free-living adults are aware of this, with many stating that their reasons for taking part in physical exercise stem from weight management and reducing the negative impact of aging (Allender et al., 2006). And yet, given this knowledge, many adults do not take part in any physical activity at all, and many more fail to meet the recommended guidelines (Harris et al., 2011; Ladabaum et al., 2014). Again, the reasons for this are multi-faceted, but include a lack of confidence (Allender et al., 2006), time pressures (Welch et al., 2008;

Sequeira et al., 2011), and a lack of enjoyment (Ekkekakis et al., 2008). All of these factors appear to contribute to poor uptake of, and adherence to, exercise training programme, with this poor uptake and adherence a driver of the increased obesity and chronic disease rates. Enhancing exercise adherence is, therefore, a potentially major aspect of improving population health. This section proposes the idea of exercise response efficiency, whereby individuals are matched to the exercise training modality most likely to deliver the greatest improvements in fitness in the shortest amount of time. It is believed that such an outcome would be important, as rapid improvements in fitness likely increase both confidence and enjoyment, thereby further enhancing adherence, and, as a result, reducing obesity and chronic disease rates.

2. Exercise – Good for everyone, all of the time?

There are many different modalities of exercise that can be undertaken, existing on a continuum from aerobic endurance exercise to resistance exercise (Egan & Zierath, 2013), and from low to high intensity. These divergent exercise stimuli have demonstrated, wide-ranging health promoting effects, including a reduction in adipose tissue, enhancement of glucose metabolism, reductions in blood pressure, and increases in bone mineral density (Egan & Zierath, 2013). Other exercise types, such as high intensity interval training (HIIT) have similar health-promoting and weight-management effects (Shiraev & Barclay, 2012; Gillen & Gibala, 2013), although such high-intensity exercise may—but not always—reduce enjoyment and hence adherence (Ekkekakis et al., 2008; Vella et al., 2017).

Given the wide-ranging and well-established health benefits of exercise, it might be believed that exercise is good for everyone, all of the time, and that there is a reasonably standard, predictable adaptive response to such exercise. However, recent research has called into question some of these long-held beliefs. There is now a wide body of evidence suggesting that there is considerable inter-individual variation in response to an exercise training programme. For example, in the seminal HERITAGE Family Study, which explored inter-individual variation in response to a 20-week aerobic training programme, training-induced changes in VO_{2max} ranged from a reduction of approximately 100 mL O_2/min to an increase of over 1000 ml O_2/min (Bouchard & Rankinen, 2001). Furthermore, whilst the majority of participants demonstrated a reduction in heart rate (HR) response to a given workload following the training programme, approximately 100 individuals (~14% of participants) demonstrated an increase in HR response, suggesting a reduction in physical fitness. Furthermore, when analysing pooled data from six different training intervention studies, Bouchard and colleagues (2012) reported that, following exercise, 8% of participants had an adverse change in fasting insulin, 12% an adverse change in systolic blood pressure, 10% an increase in triglycerides, and 13% a reduction in high density lipoprotein—all undesired responses that potentially serve to increase the risk of disease.

Individuals demonstrating an increase in risk factors following exercise have been termed adverse responders, whilst those demonstrating no measurable improvement in a measured fitness variable have been termed non-responders. Recently, a number of researchers have explored the use of such terms skeptically (Atkinson & Batterham, 2015; Hecksteden et al., 2015; Williamson et al., 2017;

Williamson et al., 2018; Atkinson et al., 2018), suggesting that this heterogeneity in response may be (at least partly) due to measurement error and random daily variation, and may not be clinically relevant. In a recent review (Pickering & Kiely, 2018b), it was suggested that global non-responders to exercise—i.e. individuals demonstrating no beneficial effect of exercise—likely do not exist; nevertheless, when it comes to changes in disease-associated measures, such as cardiorespiratory fitness and fasting insulin, exercise—or at least specific types of exercise—appear not to have the same beneficial effects for all.

3. The causes of exercise response heterogeneity

The drivers of this inter-individual response to a training stimulus are wide and varied. Exercise response is often determined by comparing the pre- and post-intervention scores on a given measure. Inherent within any measurement are technical error and random within-subject variation; both of these are said to represent "false" inter-individual variation (Atkinson & Batterham, 2015). Conversely, drivers of "true"—that is, real—inter-individual variation can best be categorised as genetic, environmental, and epigenetic factors (Pickering & Kiely, 2017a). As an example of the impact of genetic variation, a single nucleotide polymorphism (SNP) within *ACTN3* has been demonstrated to affect the adaptive response to resistance training in elderly individuals (Delmonico et al., 2007). An example of the environmental influence on exercise adaptation is that of stress; individuals who have increased life stress may exhibit a reduced adaptation to a training stimulus (Bartholomew et al., 2008). Finally, epigenetic modifications and translational control mechanisms, such as microRNAs, may modulate the adaptive response to exercise (Davidsen et al., 2010), either by making specific points within DNA more accessible to translation, or exerting control over messenger RNA by either inhibiting translation or causing degradation before translation occurs (Nielsen et al., 2014).

4. A lack of exercise response is both modality and measurement specific

The existence of non- or low-responders to exercise is potentially troubling, as it suggests that a sub-group of people may gain no benefit from exercise training. However, it appears that such a low response to exercise is both modality and measurement specific (Pickering & Kiely, 2018b), suggesting that a change in exercise training type, or the inclusion of additional measurements, may reduce the rate of exercise non-response.

A limited number of studies have explored exercise response across more than one exercise modality. Hautala and colleagues (2006) placed 73 participants through separate endurance and resistance training programmes in a randomised cross-over design, determining improvements in peak oxygen uptake (VO_{2peak}) following both interventions. There were inter-individual variations in VO_{2peak} improvements following both aerobic (range -5 to +22%) and resistance (range -8 to +16%) training, illustrating that some participants demonstrated no improvements following a given training type. However, participants with the lowest VO_{2peak} improvements following aerobic training exhibited a greater improvement in this measure following resistance training.

Furthermore, when increasing the number of measurements taken, exercise non-response appears to disappear. This was demonstrated by Karavirta and colleagues (2011), who found that, whilst a small number of participants demonstrated a negative training response in terms of VO_{2peak} or maximum voluntary contraction following a combined aerobic and strength training programme, no subject exhibited a negative response to both. Similarly, Bonafiglia and colleagues (2016) subjected individuals to both endurance and sprint interval training, determining improvements in VO_{2peak}, lactate threshold, and heart rate following training. Whilst some participants exhibited non-response to one of these measures, very few were non-responders across all three.

5. Exercise response efficiency

Given the research discussed previously, it is apparent that not everyone can demonstrate favorable adaptations to every exercise modality, all of the time. Considering the clear disease prevention, control, and treatment effects of exercise, such a finding is potentially troubling, suggesting that not everyone can maximally harness such effects—and, in turn, cannot gain the same reduction in disease risk as other individuals. Instead, it would perhaps be of greater benefit to match individuals to the type of training they are most likely to demonstrate beneficial adaptations to. At present, such an approach typically occurs through trial and error; an individual undertakes a training intervention—often lasting weeks or months—and then discovers whether they have improved or not. If they have, they may continue the intervention; if they haven't, they can try a different exercise modality. However, this approach is costly in terms of time; given that one of the cited reasons for a lack of exercise adherence are time pressures (Welch et al., 2008; Sequeira et al., 2011), such an approach may not be viable. Additionally, many people who do not currently meet exercise guidelines are anxious and unconfident regarding exercise (Allender et al., 2006); failure to demonstrate improvements may further reduce individual confidence, and reduce enjoyment, limiting the potential of that person to undertake exercise in the future.

Recent evidence suggests that exercise non- or low-response can be abated through increases in training volume, intensity, or duration (reviewed by Pickering & Kiely, 2018b); however, in high-risk populations, exercise intensity may be poorly tolerated and unpalatable (Hardcastle et al., 2014), whilst increased volumes and durations are unlikely to be successful due to a perceived lack of time to exercise (Welch et al. 2008; Sequeira et al., 2011). Instead, by matching individuals to the exercise type in which they demonstrate the greatest adaptive potential, it might be possible to:

1) Reduce disease risk factors in a shorter period of time. This is especially important given the lack of time—real or perceived—often cited as a reason for non-adherence to exercise guidelines. If larger improvements can occur in a shorter amount of time through targeted training, this would be hugely beneficial to many people.
2) Promote greater adherence to exercise. Research from the nutrigenetics field demonstrates that, when individuals are placed on a personalised dietary intervention, they are more likely to adhere to that intervention for a greater period of time (Arkadianos et al., 2007)—there is no

apparent reason why this would not be the case with exercise. Additionally, by increasing the improvements gained from exercise, the fulfilment and enjoyment experienced by the individual is likely to be increased—further promoting long-term exercise adherence.

6. How can individuals be matched to their optimal training type?

The ability to match individuals to the training type most likely to yield the greatest improvements in specific outcomes is, at present, hugely under-explored. In part, this is because it remains to be fully elucidated what variables may predict the most effective training type. From an obesity standpoint, recent work by Leonska-Duniec and colleagues (2018a, 2018b, 2018c, 2018d) has explored the effects of a number of SNPs on change in fat mass and improvements in aerobic fitness in a group of untrained female participants. Following a 12-week aerobic training programme, only 75% of participants lost fat mass, and participants with a greater number of obesity-risk alleles tended to lose less fat following training (Leonska-Duniec et al., 2018a). Other obesity SNPs, such as *LEP* and *LEPR*, which encode for leptin and its receptor, modified the change in glucose and LDL cholesterol levels following this same training intervention (Leonska-Duniec et al., 2018d), results which replicated findings from HERITAGE (Lakka et al., 2004). Similar results have been reported by Klimentidis and colleagues (2016), who found that the possession of a greater number of obesity-risk alleles was associated with a lesser reduction in fat mass following resistance training. However, at present, whilst it's clear that a variety of SNPs, such as *ACTN3* (Pickering & Kiely 2017d) and the obesity related SNPs discussed previously (Klimentidis et al., 2016; Leonska-Duniec et al., 2018d), affect the adaptive, fat loss, and health biomarker response to training, at present very few studies have attempted to utilise this information to inform training programme design.

A previous study (Jones et al., 2016) utilised a 15 SNP total genotype score to classify participants as those expected to respond better to high-volume, moderate-intensity resistance training, and those expected to respond better to low-volume, high-intensity resistance training. The participants were then randomised to receive either "matched" (i.e. training matched to their genotype score) or "mismatched" training over an eight-week resistance training intervention. Those in the matched training group experienced significantly greater improvements in a test of power and a test of endurance compared to those in the mismatched group. Furthermore, 83% of high responders to the training intervention were from the matched group, whilst 82% of low- and non-responders were from the mismatched training group. More recently, the study presented in Chapter 11 (Pickering et al., 2018) utilised a 5 SNP genetic test to predict the magnitude of improvements in Yo-Yo test score—a measure of aerobic capacity—in a group of youth soccer players. Participants in possession of a greater number of SNPs thought to be associated with larger improvements in aerobic capacity did indeed demonstrate such improvements, whilst those predicted to demonstrate smaller improvements also did so. These findings suggest that genetic information may hold promise in matching individuals to the training type most likely to elucidate the greatest adaptive response.

Similar results have been reported around aerobic training. Timmons and colleagues (2010) discovered a specific molecular signature, comprised of 29 RNAs expressed within muscle prior to a training intervention, which predicted the improvements in VO_{2max} demonstrated following that training intervention. Similarly, Davidsen et al. (2011) uncovered four miRNAs that were differentially expressed between low- and high-responders to a twelve-week resistance training programme, adding further to the promise of the matching of individuals to their most responsive training type in the future.

At present, tentative research suggests that a combination of genetic and miRNA markers at baseline may be able to predict the magnitude of training response to a given intervention (Timmons et al., 2011; Davidsen et al., 2011; Pickering et al., 2018). This raises the potential for those individuals expected to demonstrate a lower response to a specific intervention to undertake a separate intervention—one in which they are expected to demonstrate a larger improvement, and hence derive increased health benefits. Early research suggests that genetic information may assist in the matching of optimal training type to each individual (Jones et al., 2016), although significantly more research is required to confirm and expand on these early promising findings.

7. Conclusion

This part of the chapter has speculated that, by matching individuals to the type of training they are most likely to see the greatest improvements from, it may be possible increase the protective effects of exercise against disease and promote long term exercise adherence. Such an outcome, it is proposed, represents a type-efficient method to best maximise the health of at-risk populations. Early research suggests that genotype-matched training (Jones et al., 2016) can enhance training adaptations, and that a number of markers, including miRNA (Davidsen et al., 2010) and genetics (Timmons et al., 2011; Pickering et al., 2018), can predict the magnitude of training response prior to an intervention taking place—allowing for modifications to be made prior to a lower than optimal adaptation occurring.

However, such an approach requires greater investigation before it can be integrated into disease control and treatment plans, with the early findings requiring replication, and further studies needed to explore the efficacy of such an approach on training-induced outcomes and adherence in at-risk populations. Additionally, the cost of genetic and miRNA testing may make such an approach cost-prohibitive, at least in the short-term, to publicly funded health bodies, or lower socio-economic status individuals wishing to pursue such an approach privately.

Nevertheless, such an approach might be prudent in future when targeting the most high-risk individuals in a population. Given the wide-ranging and well-established health benefits of exercise on obesity and disease risk and treatment, but with the current poor uptake of exercise programmes, this approach may serve to both increase adherence and results. Given the increasing numbers of individuals with obesity and chronic disease across the globe, along with declining physical activity rates, such an approach represents a potentially useful tool to attack such issues.

PART THREE – GENETICS & DIET CHOICE

Whilst exercise is a potentially highly effective treatment for obesity (Epstein & Goldfield, 1999), its results can be enhanced via improved dietary management. Furthermore, dietary mis-management, including the overconsumption of total calories, and specific macronutrient types, can serve to increase the risk of obesity (Bray & Popkin, 1998), along with that of other diseases, such as type-II diabetes (Schulze et al., 2004) and cardiovascular disease (Siri-Tarino et al., 2010). A variety of different SNPs have been implicated in modifying the interaction between macronutrients and disease risk, with several genotypes associated with an increased risk of developing obesity (Rankinen et al., 2006; Sonestedt et al., 2009; Corella et al., 2011); indeed, the heritability of BMI is estimated at up to 70% (McPherson 2007). An example of a gene strongly implicated in obesity is that of *FTO*, the fat mass and obesity related gene (Sonestedt et al., 2009; Sonestedt et al., 2011). Here, individuals in possession of a risk allele of a common SNP within this gene (rs9939609) are on average 3 kg heavier and 1.67 times more likely to be obese than those without any risk alleles (Frayling et al., 2011). This is especially true when their overall fat intake is relatively high (Sonestedt et al., 2011), with saturated fat intakes appearing to be main driver of this relationship (Corella et al., 2011). Similarly, variation with *TCF7L2* has been shown to modify the effects of carbohydrate intake on type-II diabetes risk, with risk allele carriers shown to be around three times more likely to develop the disease if they were in the highest tertile for high glycemic load (GL) carbohydrate intake compared to those individuals possessing no risk allele (Cornelis et al., 2009). However, if risk allele carriers were in the lowest tertile for high GL carbohydrate intake, they had no increased type-II diabetes risk, demonstrating the potential of genetic information to inform diet choice.

As a result, a number of studies have explored the potential utility of genetic information, most commonly in the form of a total genotype score, to guide diet choice (Arkadianos et al., 2007; Nielsen et al., 2012), with some success. Arkadianos and colleagues (2007) assigned participants at an obesity clinic to receive either a nutrigenetic diet (i.e. a diet "matched" to an individual's genotype), or a standard control diet. Initially, both groups lost fat; however, after 300 days, those in the nutrigenetic diet were more likely to have maintained this fat loss compared to the control group, who had instead started to regain weight. As such, the authors suggested that genetically-matched diets diet increase subject compliance. This hypothesis was further supported by the results of the DIETFITS Randomised Clinical Trial (Gardner et al., 2018). Here, overweight participants were randomised to receive either a high-fat or a high-carbohydrate diet, which were matched for calories. The participants received intensive lifestyle coaching and had regular meetings with a dietician and support group. They also underwent genetic testing for three SNPs thought to influence fat loss efficiency, but were not informed of their results during the trial. The results of DIETFITS demonstrated no significant difference between high-fat and high-carbohydrate diets when calories were matched, and no effect of genotype on the efficacy of the diet. This suggests, as hypothesized by Arkadianos and colleagues (2007), that the positive outcomes of a genetically matched diet are due to adherence; in the DIETFITS study, the high level of support and

education, and careful controlling of calories, was not necessarily indicative of free-living individuals not enrolled in a clinical trial, where motivation and adherence are likely more important.

Based on these early findings, it appears that, in free-living individuals, the use of a genetically matched diet is associated with enhanced outcomes, in terms of changes in both fat mass and cardiometabolic health markers. As these results are not seen when participants receive intensive, in-person coaching and support, it appears that the main benefit of such a personalised approach is linked to increased dietary adherence.

Chapter summary

This chapter has explored how genetic information might be used to enhance both public and individual health. Part One demonstrated how a common polymorphism in *ACTN3*, with consistent and replicated effects on muscle physiology in elite athletes, affects the healthy aging process. Building on these findings, it was suggested that knowledge of *ACTN3* genotype, in combination with the genotype of other SNPs affecting muscle function with age, could be used to motivate individuals to undertake physical activity interventions aimed at supporting the healthy aging process, as well as informing the design of such interventions. Part Two discussed how obesity—possibly the single biggest public health issue in the Western World—could potentially be better controlled through the use of targeted and efficient exercise. This led to the promotion of the idea of "exercise response efficiency" as a method to match at-risk individuals to the type of training they are most likely to derive the largest adaptations to, in the shortest amount of time, and hence achieve the greatest reduction in risk. Finally, Part Three discussed the interaction between genotype and dietary intake on various indices of health, reporting results demonstrating the efficacy of genetic testing in matching individuals to their optimal diet type in terms of treating obesity.

As a result, based on the three aspects discussed in this chapter, it appears that there is the clear potential for genetic information to be utilised in the management of public health across the lifespan. Outside of the physiological effects of training and dietary interventions matched to genotype, the adherence and compliance outcomes also show strong promise, suggesting that the positive effects from a temporary intervention can be converted into lifelong behavioral change. These preliminary findings suggest that there is scope for future research in this field to explore the use of genetic information in health and wellbeing management, not just in terms of its physiological outcomes, but also from the standpoint of long-term compliance. In doing so, there is the potential to affect real positive change in those individuals demonstrating the greatest risk for various disease states, which in turn could have massive implications for the development and maintenance of optimal population health.

CHAPTER 14 – THE IMPLEMENTATION OF GENETIC INFORMATION WITHIN A PERSONALISED TRAINING FRAMEWORK

Chapter preface:

The theme of this thesis is to explore the potential utility of genetic information in elite sport. An important consideration is that, even if genetic information holds utility in elite sport, it should not be used as a standalone, but instead as an additional piece of information, layered on top of existing metrics and data. This chapter presents a framework for the use of genetic information as part of a personalised training approach in elite sport. This paper has been previously been published as a review article (Pickering & Kiely, 2019a).

1. Introduction

Throughout this thesis, a common exploratory theme has been whether genetic information may hold utility within an elite sport programme, and be used to enhance performance. The thesis has primarily focused on elite sport because this tends to be where innovations can take hold; often, the budgets of the teams and individuals at the highest level are greater than at lower levels, and there is the potential for enhanced buy-in, from players and staff, who are highly motivated and therefore more likely to adhere to any given intervention—in this case, genetic testing.

Early on in this thesis, a critical examination of the current approach to research within the sporting genomics sphere suggested that the vast majority of research in this field is focused on explaining the differences between two groups of performers, either to explain elite athlete status, or to explain differences in exercise adaptation in response to a training stimulus (see Chapter 4 for more detail). Whilst this information holds some utility, its usefulness is, arguably, limited; a first-team player at an elite sporting club doesn't require a genetic test for talent identification (and, as shown in Chapter 12, such a test likely wouldn't be valid anyway), and their coaches have likely already experienced the heterogeneity in response to an exercise stimulus. Instead, there needs to be somewhat of a paradigm shift in exercise genetics research, enabling a better understanding of how this information might be utilised to enhance training programme design. For example, whilst there is a large body of research demonstrating that both *ACTN3* and *ACE* modify the response to strength training (Delmonico et al., 2007; Wagle et al., 2018), there are far fewer studies exploring how to utilise this information to enhance the response of different genotypes. A theoretical paper (Kikuchi & Nakazato, 2015) first explored this, suggesting that *ACTN3* R allele carriers—those expected to demonstrate the greatest response to high-load resistance training—should prioritise high-load resistance training, with an emphasis on eccentric loading, along with high-intensity interval training (HIIT). Conversely, those with the XX genotype were suggested to be better placed to undertake low-load, high-volume resistance training, minimising eccentric loading (to which they have an increased susceptibility for muscle damage [see Chapter 6]), and undertaking longer, lower-intensity aerobic training.

The first study to attempt to directly test this hypothesis was published in 2016 (Jones et al., 2016). Here, participants underwent genetic testing in order to establish their genotypes for 15 separate single nucleotide polymorphisms (SNPs) thought to influence the adaptive response to resistance training. Using a total genotype score (TGS) approach, the participants' genotypes at each SNP were given a score between 0 and 4, allowing the calculation of whether they would be expected to respond better to "power-biased" (high-load, low-volume) or "endurance-biased "(moderate-load, high-volume) resistance training. The participants were then randomly assigned to receive either genetically matched or mismatched training. The results showed that those undertaking genetically matched training—i.e. power-biased participants undertaking power-biased training, or endurance-biased participants undertaking endurance-biased training—achieved around three times the magnitude of performance improvement in countermovement jump (CMJ) height and Aero3 tests. The results of this study suggested, for the first time, that genetic information could be used to enhance training adaptations.

Taken together, the results of this earlier work, both theoretical (Kikuchi & Nakazato, 2015) and applied (Jones et al., 2016), along with the theoretical and applied aspects of this thesis, suggest that the ability to begin to utilise genetic information to enhance elite sport performance is close. To continue down this road, researchers will have to seek to further bridge the gap between lab and field, focusing on the practical applications of their work, and supporting coaches and athletes in their quest to enhance performance (Buchheit 2017). Specifically, it is important to better understand how a variety of emerging technologies—not just genetic information—can be utilised to assist coaches in getting closer to a definitive answer to the following questions:

1) To what training will my athlete best respond?
2) How well is my athlete adapting to training?
3) When should I change the training stimulus (i.e., has the athlete reached their adaptive ceiling for this training modality)?
4) How long will it take for a certain adaptation to occur?
5) How well is my athlete tolerating the current training load?
6) What load can my athlete handle today?

This chapter aims to explore novel methods which, when used alongside existing technologies, will hopefully help coaches gain answers to the above questions. This should assist in the decision-making process, allowing for the targeted use of emerging technology to guide such decisions, and contribute to an enhanced understanding of the way in which each individual responds to exercise training, both in terms of adaptation and fatigue.

2. A personalised medicine approach to performance

The announcement of the Human Genome Project (HGP) lead to the belief that it would soon be possible to understand the genetic and molecular underpinnings of disease, and, in turn, be able to develop personalised treatments for individuals to combat such diseases. This, coupled with the

decreasing costs associated with genome sequencing, lead to the US National Human Genome Research Institute to formalise a 20-year plan aiming to translate the insights, from both the HGP and early pilot studies, into medical breakthroughs (Green & Guyer, 2011; Manolio & Green, 2014). The spotlight was further shone on the promise of precision medicine by President Barack Obama, who, in his 2015 State of the Union address, proposed a vision for a Precision Medicine Initiative within the US (Ashley 2015; 2016).

The precision medicine movement has had some success. For example, an enhanced understanding of the genetic mutations within *CFTR* which cause Cystic Fibrosis (CF) has improved treatment for many sufferers. Here, patients can now be stratified into subgroups based on their *CFTR* genotype; the mutation type determines the effectiveness of the drug ivacaftor in the treatment of CF. In the ~85% of patients expected to see a reduced effectiveness of ivacaftor, a second drug, lumacaftor, can be given in combination, which appears to enhance treatment effectiveness (Wainwright et al., 2015; Ashley, 2016). Similarly, it is understood that genetic variants help explain susceptibility to diseases (Hofker & Wijmenga, 2009; Yan et al., 2009) allowing for more personalised, targeted advice to be given to those with the increased risk (Pine et al., 2016).

Alongside disease prediction and management, an understanding of genetic variation has been used to personalise drug treatments through the field of pharmacogenomics (Relling & Evans, 2015). Here, information on genetic variants known to influence drug pharmacokinetics or pharmacodynamics is utilised to guide drug selection and dosage (Relling & Evans, 2015), such as the success seen in genotyping both *VKORC1* and *CYP2C9* to optimise the dose of warfarin (International Warfarin Pharmacogenetics Consortium, 2009). This information can also be used to guard against adverse reactions to drugs (Yip et al., 2015; Zhang & Sarkar, 2018); for example, variation in *CYP2D6* leads to an increased sensitivity to codeine, requiring an alternative drug to be used (Ashley 2016). Additionally, within the oncology sphere, there is the potential to sequence individual patient tumors, and utilise this information to guide treatment options (Thomas et al., 2014; Damodaran et al., 2017), such as the provision of larotrectinib in TRK fusion-positive tumors (Drilon et al., 2018). Alongside genomics and pharmacogenomics, precision medicine has expanded to utilise other "-omes" and "-omics" technologies (Caudle et al., 2010; Hasin et al., 2017), such as an understanding of the microbiome in human health and disease, epigenetics, transcriptomics, proteomics, and metabolomics (IHMP, 2014; Birnet et al., 2016; Hasin et al., 2017).

Despite the potential promise of precision medicine, such an approach has yet to fully reach its potential, and has been subject to a range of criticisms regarding its effectiveness (Taylor-Robinson & Kee, 2018). Nevertheless, it remains a tantalising proposition for the integrated health management of patients, and, as research progresses and challenges are overcome, will doubtlessly assist in the prevention and treatment of a number of diseases (Ashley 2016). Additionally, the precision medicine framework has been proposed as a future method to improve both health and performance in athletes (Montalvo et al., 2017). In this case, both genetics and genomics, in partnership with additional -omic technologies, could be used to detect underlying conditions that may alter athlete health, such as Hypertophic Cardiomyopathy (HCM) (Maron et al., 2012), injury risk (e.g. *COL5A1* [Posthumus et al.,

2009c]), exercise adaptation (see Chapter 2 for more details), nutritional requirements (Ordovas et al., 2018; Guest et al., 2019) and ergogenic aid use (see Chapter 5 for further information).

The upcoming sections detail how some of the methods inherent within the personalised/precision medicine process may be utilised within the elite sports sphere in the future, allowing for the development of the personalised training process.

3. Novel markers of exercise adaptation and recovery

3.1 Epigenetic modifications—novel markers of exercise adaptation and fatigue

As detailed in Chapter 2, epigenetic modifications act to regulate genetic expression. Epigenetics can be very broadly defined as changes in genetic expression that occur without a change in the underlying genetic code. There are numerous different epigenetic changes that can occur, of which three are most well studied; DNA methylation, histone modifications, and microRNAs (miRNA) (Ehlert et al., 2013; Ling & Ronn, 2014; Moran & Pitsiladis, 2016). Epigenetic modifications have the potential to be heritable (Voisin et al., 2015), but also may be both malleable and transient (Voisin et al., 2014), and have been proposed as potentially important modifiers of exercise adaptation (Polakovicova et al., 2016; Hakansson et al., 2018).

3.1.1 Methylation

DNA methylation refers to the addition of a methyl ($-CH_3$) group to a cytosine (C) DNA base. The methyl group reduces the availability of the cytosine base to the DNA transcription machinery, which therefore limits the transcription of that particular section of the gene. Whether this is positive or negative is context specific, depending on whether transcription of that specific gene is desired. For example, methylation of *PPARGC1A*, a gene involved in mitochondrial biogenesis, is associated with an increased risk of type-II diabetes (Ling et al., 2008). Conversely, methylation of a number of cancer promotor genes is likely positive, reducing the risk of the disease (Voisin et al., 2014). Regular exercise is able to both methylate disease-associated genetic variants, and de-methylate (i.e. remove the methyl group) genes associated with positive exercise adaptations (Voisin et al., 2014; Ling & Ronn, 2014; Pareja-Galeano et al., 2014). This relationship is fluid and transient, with methyl markers associated with inactivity removed when the individual undertakes exercise training (Ling & Ronn, 2014). Recently, Seabourne and colleagues (2018) demonstrated that skeletal muscle has an epigenetic memory, with acute exercise producing methylation patterns that are maintained through a period of inactivity, and which appear to subsequently enhance later adaptations to resistance training.

As such, there is the possibility of utilising methylation patterns as markers of current status, providing insight to the training history of athletes. As research in this area develops, it should be possible to gain an understanding as to what the implications of specific methylation patterns are, such this information could be used to determine the responsiveness of a given athlete to a stimulus. Additionally,

aberrant methylation patterns could be identified, and training programmes designed to remove those patterns, potentially enhancing subsequent exercise adaptation.

3.1.2 Histone modifications

DNA is wrapped around structural proteins called histones, giving it a tightly coiled structure. The tightness of these coils makes the individual bases poorly accessible to the various different transcription factors and enzymes requires to transform the raw code of DNA to the required protein. To combat this, the body has evolved a method for various different stimuli—including exercise—to better access its DNA when required; that of histone modifications. Here, the histone proteins are acted on to allow the DNA to un-coil, making it more accessible for translation to the required protein. This primarily occurs via the addition of an acetyl group to the histone protein, which is catalysed by the histone acetyltransferase (HAT) enzyme group (McKinsey et al., 2001). In turn, the acetyl group is removed by histone deacetylase (HDAC) (McKinsey et al., 2001).

Given their fundamental role to play to gene transcription, and, given that transcription of genes is a crucial aspect of exercise adaptation (Egan & Zierath, 2013), it's clear that both HATs and HDACs have the potential to modify the response to exercise. This has been studied in mice models, where an increase in a specific HDAC, HDAC5, blunted the expected increase in type-I fibres following aerobic training (Berdeaux et al., 2007). Other studies have demonstrated how concentrations of both HATs and HDACs may alter muscle plasticity; acetylation of the histone H3, for example, has been linked to alterations in the expression of myosin heavy chain genes, which in turn potentially alters muscle fibre type (Pandorf et al., 2009). As a result, the monitoring of HAT/HDAC concentrations may assist in understanding the training response. If there is an increase in those HATs/HDACs associated with an increase in type-I fibre following training, and the athlete is a sprinter, it would seem logical to modify the training stimulus to instead provide a more optimal adaptation.

Of the three major epigenetic modifications, histone modifications are perhaps the least well understood, in part due to the fact that they are highly site-specific, so changes occurring within the muscle would require a biopsy. Given the largely transient nature of histone modifications, frequent biopsies would be required, a process which often is not feasible, especially in elite athlete cohorts.

3.1.3 miRNA

Traditionally, it was believed that RNA served as an intermediate step between DNA and the proteins produced; in this early model, RNA, in the form of messenger RNA (mRNA) was produced from DNA during transcription, with the mRNA then being transported to the ribosome for production of the required protein. However, the results of ENCODE (Encyclopedia of DNA Elements) suggested that, whilst ~75% of the human genome is transcribed into RNA, only a very small proportion (~3%) is directly involved in the creation of proteins (ENCODE Project Consortium 2012). This suggests that the vast majority of RNA is not involved in the creation of protein, but instead, in some cases, may alter the translation of proteins by controlling mRNA (Bartel, 2004; Polakovicova et al., 2016). miRNAs play a

role in modulating metabolism and inflammation, which in turn may impact exercise recovery and adaptation (Polakovicova et al., 2016). As such, they represent potentially important biomarkers in the personalised training process.

The role of miRNAs in adaptation to resistance training has been explored in a few studies. Two miRNAs, miR-1 and miR-133a, are expressed during skeletal muscle hypertrophy (McCarthy & Esser, 2007). Importantly, differences in miRNA concentrations may be able to predict exercise training response. Davidsen and colleagues (2011) reported that high- and low-responders to a resistance training programme differentially expressed four miRNAs, with three (miR-378, miR-29a, miR-26a) downregulated in low responders, and one (miR-451) upregulated in low responders. Similarly, Horak et al. (2018) demonstrated that baseline levels of miR-93 represented an independent predictor of improvements in isometric leg extension following a resistance training programme.

miRNAs have also been implicated in modifying the response to aerobic endurance training. Nielsen and colleagues (2010) demonstrated that specific miRNA concentrations altered in response to both an acute aerobic training session, as well as a longer-term (12-week) training programme, a result which has been replicated (Russell et al., 2013). Aoi and colleagues (2013) demonstrated that a specific miRNA, miR-486, was significantly decreased following both acute and chronic endurance training when compared to baseline, and the ratio of this change was negatively correlated with changes in VO_{2max}. Additionally, Domanska-Senderowska et al. (2017) found a correlation between miR-29a and VO_{2max} training improvements in a group of soccer players. miRNAs may also be useful in assessing baseline fitness, with three (miR-210, miR-21, and miR-222) associated with lower VO_{2max} (Bye et al., 2013).

Furthermore, the manipulation of training variables has been demonstrated to affect miRNA levels. As an example, Schmitz and colleagues (2018) reported that 4 x 30s high-intensity sprint running repetitions significantly increased both miR-222 and miR-29c levels, whilst 8 x 15 second sprints did not. Both of these miRNAs are associated with adaptations to exercise; miR-222 plays a role in exercise-induced cardiac growth (Liu et al., 2015), whilst miR-29c is a modulator of cardiac muscle remodeling (van Rooij et al., 2008). Additionally, miRNAs appear, at least in some cases, to be sensitive to exercise dose, plateauing if there is insufficient progression (Schmitz et al., 2017).

miRNAs also hold potential as markers of exercise load. As an example, Gomes and colleagues (2014) reported that three miRNAs (miR-1, miR-133a, and miR-206) were significantly elevated following a half marathon when compared to baseline. Further research examined the differences in miRNA release following 10km, half-marathon and marathon runs (de Gonzalo-Calvo et al., 2015), with the extent of miRNA increases distinct between the distances. These specific miRNAs were associated with inflammation, suggesting that practitioners may be able to better understand the individual inflammatory response to exercise, allowing for more personalised recovery processes to be put in place. Recently, Hakansson and colleagues (2018) identified differences in miR-29a-3p (which was also identified by de Gonzalo-Calvo et al., 2015) and miR-495-3p expression between elite athletes and peripheral artery disease patients following exercise, suggesting that these miRNAs may hold promise as markers of muscle recovery following exercise.

As such, the evidence suggests that the monitoring of miRNA concentrations, both before and during an exercise programme, may hold utility. The measurement of concentrations prior to beginning an exercise programme may be able to identify high- and low-responders to that intervention (Davidsen et al., 2011; Horak et al., 2018), allowing for the modification of the subsequent training programme. Similarly, the monitoring of miRNA concentrations during the training programme may act as a real-time monitor of adaptation, with increases or decreases in specific miRNAs associated with a particular training response (Schmitz et al., 2018). In time, as research in this field progresses, it may be possible to match specific miRNAs to a specific molecular process; here, coaches will be able to understand whether the desired training effects—such as an increase in mitochondrial biogenesis—are actually occurring. Early evidence suggests that this innovation is close; the identification of miR-222 and -29c as drivers of cardiac adaptations following exercise illuminates the potential utility of monitoring the concentrations of these miRNAs—should an individual not see an elevation in these miRNAs, then training intensity/duration may have to be modified to elicit such a change (Schmitz et al., 2018). Additionally, lower concentrations of miR-33 are associated with greater activation of AMPK following aerobic training (Davalos et al., 2011), and miR-29b alters PGC-1α production (Wang et al., 2008)—both molecular signals for mitochondrial biogenesis—again demonstrating how real-time monitoring of miRNA concentrations could allow coaches to understand the specific adaptations an exercise is stimulating. Regular monitoring of miRNAs also has the potential to act as a marker of adaptation, as increases in specific miRNAs appear to be blunted when exercise dose is not progressed within a training programme (Schmitz et al., 2017). Taken together, the evidence suggests that miRNAs have the potential to be utilised as biomarkers of training response (Baggish et al., 2011; Zacharewicz et al., 2013), both in terms of adaptation and recovery. However, at present, one major limitation to the use of miRNAs as biomarkers is a lack of uniformity in response across studies; very rarely has a single miRNA been shown to have a universal response to a type of exercise (Fernandez-Sanjuro et al., 2018). For example, whilst increases in miR-1 and miR-133a have been shown following endurance exercise (Mooren et al., 2014; Baggish et al., 2014; Clauss et al., 2016), other studies have found no such increase (de Gonzalo-Calvo et al. 2015). Further research will need to elucidate whether the miRNA response to exercise is heterogenous (and potentially caused by heterogeneity in individuals [Fernandez-Sanjurjo et al., 2018])—limiting their use as exercise biomarkers—or if some commonalities can be found.

3.1.4 Utilisation of epigenetic markers within training programmes

As discussed above, the three major epigenetic modifications hold potential utility for a role within the personalised training process. Of these, perhaps the most promising are miRNAs, which have the potential to serve as markers of responsiveness to a training programme prior to that programme being undertaken (Davidsen et al., 2011), allowing for the coach to match the athlete to the required exercise type. miRNAs also hold value as a real-time marker of exercise adaptation (Domanska-Senderowska et al., 2017), allowing for a change of stimulus to be applied at the most optimal time point, and as a marker of fatigue status (Hakansson et al., 2018), allowing for daily changes in training load and volume. Methylation markers have the potential to act as markers for previous training exposure (Seabourne et al., 2018), as well as giving guidance as to the current adaptive potential of an athlete at a given time

(Terruzzi et al., 2011), allowing for the required stimulus to be provided to the athlete. Finally, histone modifications may serve to allow the coach to better understand which stimulus provides which adaptive signals within each individual athlete, again allowing for a highly targeted approach to sports training.

3.1.5 Practical perspectives

Perhaps the biggest issue facing the provision of epigenetic modifications within an exercise training context is that such changes are often both tissue specific and transient (Lokk et al., 2014). As a result, the accurate determination of epigenetic changes requires the sampling of the specific tissue, such as skeletal muscle, which can be both invasive and traumatic, and hence not palatable to high level athletes. Additionally, the samples would have to be taken immediately after exercise for accurate analysis to occur. As epigenetic modifications can be both fast acting and temporary, frequent testing for such modifications would likely have to occur, increasing the cost and reducing the practicality of such technology.

However, the collection of saliva for the profiling of DNA methylation holds promise (Langie et al., 2017), with methylation sites in saliva concordant with methylation within the target tissue for some specific biomarkers. At present, this has yet to be explored within an exercise setting but, if salivary DNA methylation profiling for exercise-related modifications becomes feasible, it will remove a substantial barrier to entry for methylation profiling within elite sport.

3.1.6 Section summary

Figure 19 acts as a brief summary of the impact of epigenetic modifications on exercise adaptations and fatigue. Here, an exercise training session elicits adaptive and fatigue-inducing effects, both of which are partially controlled via epigenetic modifications. These epigenetic modifications in turn have a feed-forward effect to the next training session, modifying performance, adaptation, and fatigue response to that session.

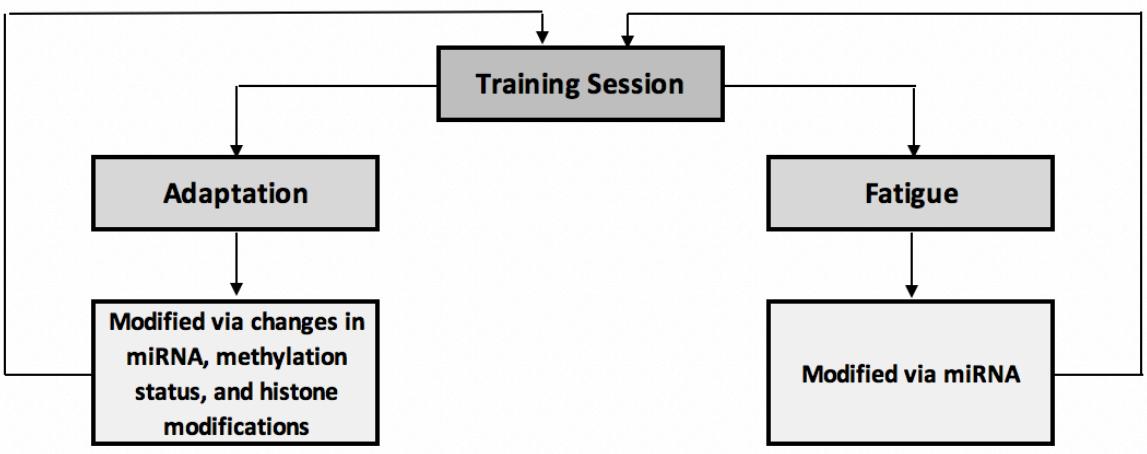

Figure 19 – Summary of epigenetic influences on training-induced adaptation and fatigue

3.2 Cell-free DNA (cfDNA): a novel marker of exercise adaptation?

Circulating cell-free DNA (cfDNA) refers to the presence of DNA fragments within the blood (Breitbach et al., 2012). At rest, small amounts of cfDNA are present in the blood stream; these concentrations have been demonstrated to increase under both acute and chronic physiological stress, such as sepsis, trauma, cancer, and myocardial infarction (Swarup et al., 2007). This is also true of exercise. For example, following a half marathon race, mean cfDNA concentrations increased from 18 pg/μL (baseline) to 335 pg/μL (Atamaniuk et al., 2004), with similar results being demonstrated following an ultra-marathon (Atamaniuk et al., 2008). This is also true for resistance training, where increases in cfDNA have been demonstrated following a single training session (Atamaniuk et al., 2010), and within a 12-week training programme (Fatouros et al., 2006).

Whilst the mechanism underpinning the increased release of cfDNA during exercise is poorly understood, it appears that cfDNA is primarily released from cells involved in immune function (Andreatta et al., 2018). The magnitude of cfDNA concentration increases also appears proportional to both exercise intensity and duration (Andreatta et al., 2018; Haller et al., 2017), and the time course of these changes is remarkably transient, with cfDNA concentrations often returning to baseline within 24 hours, even after highly exhaustive exercise (Atamaniuk et al., 2004). As a result, cfDNA represents a potentially novel biomarker for fatigue and recovery in exercise (Breitbach et al., 2012; Andreatta et al., 2018; Haller et al., 2018). In participants who undertook a 12-week resistance training intervention, cfDNA was strongly correlated with mean training load within each 3-week training sub-block (Fatouras et al., 2006). The highest concentrations of cfDNA also corresponded to a decreased performance level, leading the authors to suggest that cfDNA was a potential biomarker for overtraining; such a finding is potentially crucial given that overtraining/unexplained underperformance syndrome is, at present, largely a diagnosis of exclusion (Lewis et al., 2015). Finally, Haller and colleagues (2018) demonstrated that cfDNA increases were proportional to, and strongly correlated with, total running distance in a group of soccer players. The 23-fold increase in cfDNA concentrations demonstrated in this study is believed to be the largest biomarker increase reported following acute exercise training, suggesting a greater sensitivity than more traditional markers, such as lactate and CRP. Furthermore, the correlations between cfDNA and RPE are stronger ($r=0.58$) than for that of lactate and RPE ($r=0.32$), again demonstrating its potential utility as an exercise load biomarker (Haller et al., 2017).

The collection of samples for measurement of cfDNA is relatively straightforward, requiring a small amount of blood to be collected from a capillary (Andreatta et al., 2018), which can easily be achieved through a finger prick. As such, the use of cfDNA as a biomarker of exercise load, recovery, and overtraining is highly promising, especially given the evidence suggesting that it is more sensitive than traditional biomarkers of training load (Haller et al., 2018); as a result, its use could represent an enhancement of current practice.

4. The microbiome, exercise, and elite performance

The human gut plays host to more than 100 trillion micro-organisms (Li et al., 2014), which are collectively termed the microbiota. The roles these micro-organisms play are multifaceted, assisting in the digestion of food (Hsu et al., 2015), along with the production of nutrients such as vitamin K_2 (Marley et al., 1986), the neutralisation of pathogens and carcinogens (Nicholsen et al., 2012), and regulation of the immune system (Nicholsen et al., 2012). More recently, research has shown that the microbiota influence neurotransmitters, such as dopamine, via the gut-brain axis (Stilling et al., 2014; Clark & Mach, 2016). Additionally, it has been suggested that the microbiota may assist in the control of both the inflammatory response and oxidative stress during endurance exercise (Mach and Fuster-Botella, 2017).

Because the microbiome is modifiable by both diet and exercise (Clark et al., 2014), knowledge of the current composition of an individual's microbiome holds promise in guiding interventions. Currently, such interactions are poorly understood; whilst it is understood that specific dietary changes, such as an increase in protein (Moreno-Perez et al., 2018) or carbohydrate (Chassard & Lacroix, 2013), modify the microbiome, the effectiveness of specific changes through targeted interventions has not been tested. Additionally, whilst it is clear that diversity of the microbiota is important, with elite athletes tending to have increased diversity compared to non-athletes, and active individuals demonstrating an increased diversity compared to inactive individuals (Clark et al., 2014), it is not currently possible to offer more in-depth advice than "be active". However, as knowledge in this area increases, it appears feasible that regular monitoring of an athlete's microbiome will be able to inform dietary interventions targeted at enhancing immunity, substrate use during exercise, neurotransmitter response—which may assist in stress management—and post-exercise recovery. Accordingly, this represents a promising aspect of the personalised medicine approach to performance management in elite athletes.

5. Pharmacogenomics – personalised sports nutrition?

Pharmacogenomics refers to the identification of genetic variants that modify the effects of a given drug, most commonly through alterations in pharmacokinetics (such as the metabolisation of that drug), or pharmacodynamics (such as variation in the drug's receptor) (Relling & Evans, 2015). Chapter 5 introduced a specific example of this; that of caffeine. Here, genetic variation in *CYP1A2*, the gene encoding for cytochrome P450 1A2, affects caffeine metabolisation speed (Gu et al., 1992). The evidence suggests that individuals with the AA genotype at a specific SNP—rs762551—within this gene metabolise caffeine quicker than C allele carriers (Sachse et al., 1999), an example of pharmacokinetics specific to sports nutrition. Additionally, variation in *ADORA2A* appears to modify the binding characteristics of caffeine to the adenosine-2a receptor, which in turn alters caffeine's effects on downstream dopamine transmission (Fulton et al., 2018); an example of pharmacodynamics.

Knowledge of the differences in *CYP1A2* and *ADORA2A* genotype may, as suggested in Chapter 5, inform pre-competition caffeine strategies. For example, *CYP1A2* AA genotypes appear to experience greater ergogenic effects following caffeine ingestion than C allele carriers (Womack et al., 2012; Guest

et al., 2018); indeed, CC genotypes may even find some doses of caffeine ergolytic (Guest et al., 2018). Similarly, early research suggests that individuals with the TT genotype of *ADORA2A* experience enhanced ergogenic effects of caffeine (Loy et al., 2015). These SNPs also have the potential to modify habitual caffeine use (Cornelis et al., 2007), along with both anxiety (Alsene et al., 2003) and sleep disturbances (Byrne et al., 2012) following caffeine ingestion, suggesting that knowledge of genotype may enhance the decision-making process (Chapter 5).

Whilst caffeine offers the best example of pharmacogenomics within sporting contexts, a recent review (Heibel et al., 2018) demonstrated similar inter-individual variation in response to extracellular buffering agent supplementation (e.g. sodium bicarbonate). The inter-individual variation is partially determined by differences in *MCT1* genotype. *MCT1* encodes for monocarboxylate transporter 1, which influences lactate ion transport. As such, variation in this gene may modify the effectiveness of buffering agent supplementation. As research in this area evolves, it may be possible to identify those athletes expected to see a greater response to a particular supplement, as well as modifying dosage and timing of various ergogenic aids (Pickering 2018), as a means to enhance performance.

6. The integration of other "omes"

Alongside an understanding of the microbiome, genome, and epigenome, along with the utility of other markers, such as cfDNA, to act as novel markers of exercise adaptation and readiness, there are a variety of other "omes", including the transcriptome, proteome, and metabalome, which may enhance the personalised medicine approach to elite athlete preparation. At present, these aspects are poorly studied within an exercise setting, partly due to the complex technology and sampling methods required, and partly due to the vastness of all quantifiable aspects of each -ome.

The proteome is the term used to describe all the proteins expressed by the genome (Wilkins et al., 1996). Given that these proteins are the direct drivers of exercise adaptation, involved in, for example, skeletal muscle hypertrophy and mitochondrial biogenesis (Timmons, 2011), understanding the extent of protein expression in response to exercise, along with an understanding of inter-individual variation in the expression of a given protein in response to a specific stimulus, may assist in the matching of the athlete to the training programme best suited to the desired adaptation, along with their personal biology. At present, proteomic measurement can be extremely invasive, requiring a biopsy of the required tissue; this is problematic for muscles, causing trauma which may reduce exercise performance and increase this risk of infection, and is impossible (at present) for organs such as the heart (Petriz et al., 2012). As a result, the majority of the studies exploring the proteomic response to exercise are carried out in rats, hampering the ability to achieve clarity from their findings within human contexts.

Transcriptomics refers to the examination of mRNA levels genome-wide (Hasin et al., 2017), with these RNA levels in turn thought to act as a measure of genetic expression. Interestingly, there have been wide differences in measured mRNA expression within muscle between trained and untrained individuals in response to exercise (Wittwer et al., 2004; Stepto et al., 2009), suggesting that

transcriptomics may hold promise as a "predictor" of training outcomes. More recently, however, authors have suggested that the association between increased mRNA expression and increased gene expression may not be as strong as once thought (Burniston & Hoffman, 2011), and, indeed, may be due to technical error or random biological variation (Islam et al., 2019); as a result, transcriptomics may not be as useful as proteomics within the personalised medicine approach to athlete preparation.

Metabolomics refers to the measurement of multiple small molecule types that are downstream products of biochemical reactions (Hasin et al., 2017). Within the muscle, such metabolites could give insight into the type and rate of fuel being utilized, allowing for a personalised approach to sports nutrition. As an example, Starnes and colleagues (2017) reported significantly reduced α-tocopherol levels following exercise training in rats, suggesting that the maintenance of vitamin E levels around exercise may be important in attenuating post-exercise muscle damage. Metabolites linked to epigenetic modifications, such as folate in the case of methylation (Friso et al., 2002) could also be monitored; this is of importance given that lower levels of methylation are potentially advantageous following a hypertrophy-orientated training session (Terruzzi et al., 2011), again allowing for targeted, personalised nutritional practices to be recommended. Similar to proteomics and transcriptomics, measurement of the metabolome is, at present, highly invasive (Alves et al., 2015), limiting its potential applications to inform training programme design.

In summary, whilst proteomics, transcriptomics, and metabolomics hold potential promise as monitoring tools within the personalised training process, at present there are significant difficulties in utilising these technologies, given the highly invasive sample collection procedures, along with a lack of research within sporting contexts. As research in this field progresses, and sample collection techniques simplify, such an approach may become more feasible.

7. The use of technology in the personalised training process

The increasingly popular utilisation of various different technologies within sport has grown over the last twenty years, from simple global positioning systems able to determine distance covered (Wing 2018) to implantable devices able to measure force and strain on a muscle or tendon (Sperlich et al., 2017). The increased growth of technology has led to a number of recent reviews on the subject (McGuigan et al., 2013; Duking et al., 2016; Duking et al., 2018; Peake et al., 2018), with interest on using these technologies to design training that better matches competition performance (McGuigan et al., 2013; Wing, 2018), manage fatigue (Duking et al., 2017; 2018), and reduce injury prevalence (Duking et al., 2017), although the level of validation of these technologies is highly variable (Peake et al., 2018).

An in-depth overview of the various different technologies is beyond the scope of this chapter (and would likely be a thesis in itself), but it is worth considering how these various technologies could fit into the personalised training process. From the perspective of training programme design, real-time and retrospective data gained from these technologies can be used to design optimised programmes. For example, in the preparation of an elite sprinter, power, force and velocity profiles can be determined

through the use of timing gates (Haugen et al., 2018), force platforms (Mero et al., 1986), wearable technology (e.g. senor insoles [Nagahara & Morin, 2018]), smartphone apps (Romera-Franco et al., 2017), accelerometers embedded in external training aids (Cross et al., 2018), and high-speed video (Bezodis et al., 2008). In recent times, many of these technologies have been integrated into Inertial Measurement Units (IMUs), reducing the number of separate systems that require on-going administrating, and streamlining the data management process (Dellaserra et al., 2014; Marin et al., 2016). The data collected allows the coach and support team to determine the athlete's current strengths and weaknesses in relation to their preferred gold-standard model and/or competition performance data, with specific exercises developed to address these weaknesses (Morin & Samozino, 2016). Such an approach has been utilised to personalise optimal loading strategies in resisted sprint training (Cross et al., 2017). Additionally, McGuigan and colleagues (2013) discussed the use of a battery of strength tests to determine strength and weaknesses across the strength and power domain, again allowing for enhanced personalisation of the training process.

Technologies can also be utilised to quantify training load, which is useful in assessing fatigue and readiness to train (Halson et al., 2014; Campbell et al., 2017; Sands et al., 2017). This occurs via the quantification of both external (e.g. running velocity, duration and intensity; weightlifting sets, reps, and weight) and internal (e.g. heart rate [HR], heart rate variability [HRV]) loads, along with the determination of environmental aspects that might affect such loads, such as temperature and altitude (Hargreaves 2008; Born et al., 2014). This can also be the case in contact sports, where wearable technologies such as accelerometers may assist in the quantification of "contact load", which in turn has its own recovery requirements (Gabbett 2013). This information can then be used to better understand whether the training load is sufficient to promote the required adaptations and protect against injury, or too great, increasing injury risk (Blanch et al., 2016; Gabbett, 2016; Gabbett et al., 2016; Hulin et al., 2016a, 2016b).

Technologies can also be used to assist the coach and practitioner in determining readiness to train. For example, the use of a pre-training countermovement jump (CMJ) or measurement of bar velocity can assist in understanding the neuromuscular fatigue status of the athlete prior to training (Sanchez-Medina & Gonzalez-Badillo, 2011; Jovanovic & Flanagan, 2014; Gathercole et al., 2015), whilst metrics such as HR and HRV (measured via chest straps or smartphone apps) assist in understanding the athletes readiness to train (Plews et al., 2013; Buchheit 2014; Plews et al., 2014).

Furthermore, technologies are becoming increasingly ubiquitous within the athlete's non-training life, leading to the creation of the "24-hour athlete" (Sperlich & Holmberg, 2017). This includes the assessment of sleep measures, including duration and quality (Halson 2014), which, given the impact of poor sleep on performance (Leeder et al., 2012a), recovery (Bird 2013), cognitive function (Ferrie et al., 2011), and overall health (Irwin et al., 1996) is an important management metric.

Whilst it is easy to get carried away with the latest technology, it is worth keeping in mind that subjective markers of training load and athlete wellbeing, such as mood and perceived stress, have been

shown to outperform a more high-tech approach (Saw et al., 2016), demonstrating that a more targeted use of technology, along with common subjective markers, may represent the best approach at present.

8. Prediction, data mining & machine learning

With an abundance of information available to the coach, recent research has focused on being able to better utilise this information to underpin decision making via prediction, either in terms of injury risk (Kampakis 2016; Larruskain et al., 2018), post-injury recovery times (Kampakis 2013), physiology (such as muscle fibre type [Borisov et al., 2018]), training loads and fatigue (Gonzalez et al., 2017; Vandewiele et al., 2017), talent identification (McCullagh 2010), and training plans (Mezyk & Unold, 2011; Fister et al., 2015). These approaches utilise a variety of different statistical modelling techniques, including simple data analysis with a hold-out set for validation (e.g. Larruskain et al., 2018), more complex data mining techniques (e.g. Ofoghi et al., 2013), and machine learning tools (e.g. Vandewiele et al., 2017).

As the predictive ability of these various models tends to increase with both the amount and quality of data inputs, such methods represent promise as part of the personalised training process. Predictive modelling has been used in medicine with some success. For example, using a relatively simple Genetic Risk Score (GRS) of just 13 single nucleotide polymorphisms (SNPs), Ripatti and colleagues (2010) were able to identify individuals with a 70% increased chance of developing coronary heart disease. In building on a single data-type (i.e. genetic information) model, Khera and colleagues (2018) recently developed a GRS algorithm utilising 6.6 million SNPs to identify individuals with a threefold increased risk of developing coronary artery disease. Similar single data input models have been utilised in sport. For example, Borisov and colleagues (2018) utilised a 14 SNP model to predict muscle fibre type in 55 participants, with a Receiver Operating Characteristic (ROC) of 81% for professional athletes, demonstrating strong concordance with muscle biopsies. Such a finding could be very useful within elite sport because muscle biopsy testing is highly invasive, limiting its use, whilst genetic testing is non-invasive. Similarly, Larruskain et al. (2018) collected hamstring injury data over five seasons in an elite soccer team, along with genetic information. They then created a model of five SNPs, which demonstrated acceptable discriminatory ability to explain previous hamstring injury within that cohort. However, when applied to a hold-out data set used as validation, the model performed only as well as chance, demonstrating a lack of ability to predict injury.

As a result of the Larruskain and colleagues (2018) study, it is clear that the use of individual pieces of data is likely insufficient in the prediction of complex phenotypes and outcomes, such as injury, whilst it perhaps is sufficient for less complex phenotypes, as demonstrated by Borizov et al. (2018), who used genetic information to predict muscle fibre type with success. Data mining refers to the conversion of raw data—such as that collected by the various technological and testing practices utilised in elite sport—to information which can then be analysed (Ofoghi et al., 2013). Machine learning focuses on the development of algorithms to analyse that information, with those algorithms adapting and correcting themselves as the number of inputs increases (Sajda 2006). Again, these techniques have been utilised in

medicine, with success in predicting heart attack risk and breast cancer survivability (Delen et al., 2004; Srinivas et al., 2010; Soni et al., 2011). Within a sporting context, Vandewiele and colleagues (2017) developed a machine learning model that predicts the session rating of perceived exertion (sRPE) of the whole training group, allowing the coach to understand the general training load of a prescribed training session *before* it occurs. Additionally, their model predicted sRPE of individual athletes prior to training, allowing for the tailoring of individual workloads, and, with the addition of data collected within the training session (such as total distance covered), predict the post-training sRPE for individuals, allowing the coach to better understand the load of a given session and make changes to following sessions accordingly. This approach is potentially important, given difficulties in coaches and athletes accurately quantifying sRPE (Kraft et al., 2018), and has the potential to enhance training adaptations and reduce injury risk. The model itself was reasonably complex, with the implementation of environmental data (e.g. temperature and humidity), individual characteristics (e.g. age, current fitness level, muscle fibre type, previous sRPE scores), and training statistics (e.g. distance, duration, heart rate zones).

In summary, the use of various different models to predict a given outcome—such as injury risk, training load, or fatigue—holds promise in sport; however, as of yet it has not been extensively studied. The quality of any predictive model depends on the ability to have effective informative inputs, with an emphasis on collecting reliable and valid data. Genotype remains a promising input to such models, having been utilised in both disease (Khera et al., 2018) and sporting (Larruskain et al., 2018) domains. The ability to record an increasing richness of information, such as epigenetic modifications, along with better quantification of present metrics, such as training load, should assist in the production of valuable predictive models in the future, which, with the application of machine learning, will constantly evolve to increase predictive power with the increasing amounts of data being entered into the model.

9. A centralised framework for the development of a personalised training process

Having identified a number of different emerging technologies that, if the current understanding of them grows, hold potential in the development of the personalised training process, the next step is to understand their integration into a framework for their use.

This thesis has focused on the potential utility of genetic information in elite sport. What has (hopefully) been demonstrated is that genetic variation provides an influence on every aspect of elite athlete performance, including training adaptation (Chapter 2), injury risk (Chapter 7), ergogenic aid use (Chapter 5), post-exercise recovery (Chapter 11), athlete development (Chapter 8), and, potentially at some point in the future, the identification of future talented athletes (Chapters 8 and 12). Additionally, other researchers have identified the effects of genetic variation on important aspects such as skill acquisition (Jacob et al., 2018), psychological traits (Leznicka et al., 2018), and post-exercise fatigue (Del Coso et al., 2018), along with tangential factors which may impact athletic performance and preparation, such as nutrient requirements (Ashfield-Watt et al., 2002), microbiome composition (Goodrich et al., 2014), and bone health (Varley et al., 2018a). As such, it is clear that genetic influences are a fundamental

and consistent modifier of athletic preparation, the harnessing of which should enhance the preparation process.

However, genetic variation does not exist in a vacuum, and indeed it is not the only aspect affecting athletic preparation. As such, it needs to be placed in the correct context; for any single SNP, the likely effect on a given outcome is often very small. As discussed in Chapter 8, the identification of large numbers of SNPs that affect a given trait, along with the creation of Total Genotype Scores (TGS) for that trait, will likely improve the predictive accuracy of genetic information. But genetics will only ever serve as part of the picture; it allows an understanding of predispositions, which can be used to predict outcomes—and, as demonstrated, serve as a useful, but incomplete, input to statistical models (Borisov et al., 2018; Larruskain et al., 2018)—but the addition of further pieces of information, explored in this chapter, should enhance the personalisation process.

Figure 20 serves as an overview example of how these various technologies might be integrated to enhance athlete preparation. When devising a training plan, it is important to have a good idea of where the coach and athlete want to get to—i.e., what are the performance requirements of the athlete? This can be determined through the use of historic performance data, along with more complex predictions and trend analysis achievable through data mining and machine learning (Cust et al., 2018). Once an understanding of the destination has been achieved, the next step is to know where the athlete is starting from. This can be achieved by collecting baseline fitness data, along with some of the adaptive markers discussed in this chapter (e.g. cfDNA), in conjunction with health and wellness data (e.g. microbiome). This information is then used, along with the integration of exercise "predictors" such as genetics (Jones et al., 2016) and miRNAs (Timmons et al., 2011) to develop the optimal training programme, based around what the athlete is expected to adapt most favourably to. This plan should represent an initial outline, as opposed to a set prescription, given the highly variable nature of adaptation (Kiely 2018)—some, but not all, of which will be predictable from the information gained via the personalised training framework.

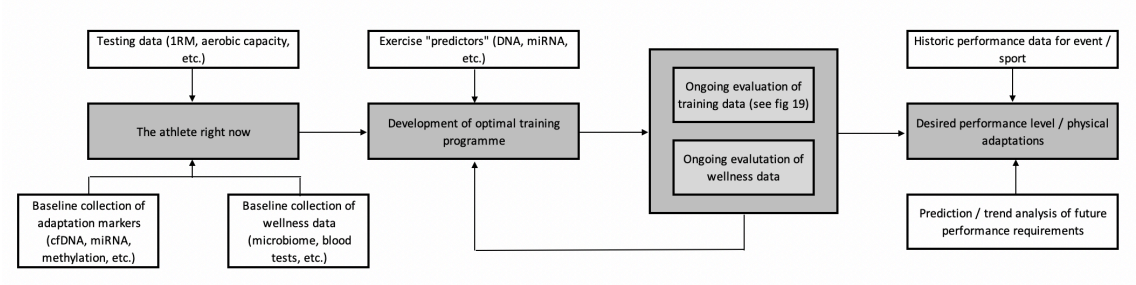

Figure 20 – An overview of the development of the personalised training process

Recognising this need for constant reformatting and reworking of the plan, figure 21 provides an overview of how the various emerging technologies discussed in this chapter can be used for the daily manipulation of training load, intensity, and stimulus, to meet the desired demands. Here, readiness to

train and the adaptive state of the athlete is determined prior to training through the integration of metrics such as sleep, HRV, readiness testing (e.g. CMJ or bar velocity), and assessment of the psycho-emotional state, along with on-going data on training load and current adaptive status determined from previous sessions. This information can then serve as an input to a statistical model which calculates the required training sRPE, similar to Vandewiele and colleagues (2017) as detailed in section 8 of this chapter. As training commences, data can be collected on aspects such as load, intensity, duration, heart rate response, environmental conditions, etc., and integrated to calculate the individual and team sRPE. Individual markers of adaptation and fatigue can then be collected from the athlete; cfDNA and miRNA can be used to assist in the quantification of fatigue and training load, with epigenetic markers used to establish whether the desired adaptations are occurring, and to what extent. Both aspects can then be compared to historical data, such as previous training load, and individual factors, such as genetics and fitness level, to understand whether the current training load is sufficient to promote adaptations, but not excessive enough as to increase the risk of injury. Similarly, in future it may be possible to use genetic information to determine a maximum threshold of possible adaptation, along with understanding what this adaptation looks like at the molecular level; this information can be compared to where the athlete is at a given point in time to determine if they have met this threshold—requiring a change in training goal—or if they can continue with the same training plan.

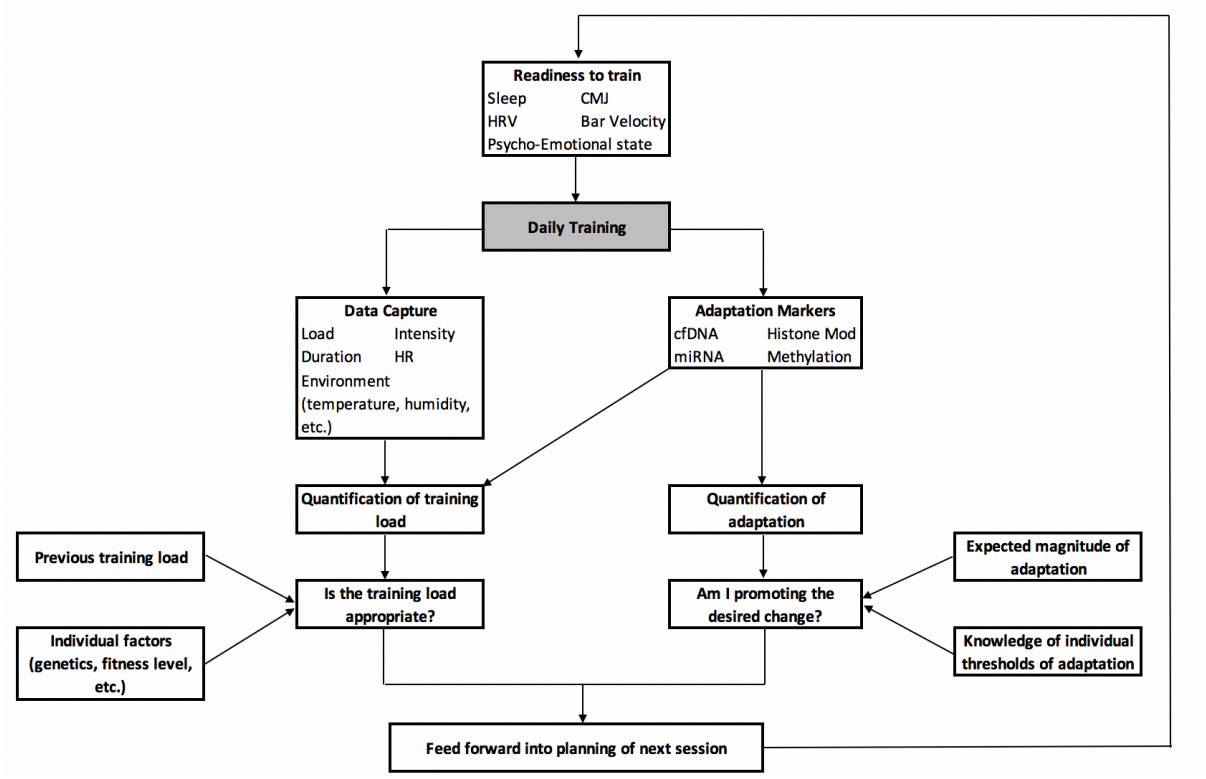

Figure 21 – A framework for the implementation of various emerging technologies to enhance daily training practice

Many additional technologies, both existing and novel, can be factored into these models as required. For example, blood testing for health markers, such as serum vitamin D, may be required; here, genetic variation can be used to predict the response to vitamin D supplementation (Gaffney-Stomberg et

al., 2017), and used to create individualised target reference values and nutrient requirements, an approach which has been highly successful in recent trials (Westerman et al., 2018). Microbiome sampling can occur less frequently, perhaps every three months, to monitor for changes. Pharmacogenomic principles can be utilised in the development of personalised sports nutrition strategies, such as the caffeine example described in section 5 of this chapter, or to guide the selection of medications required to manage issues such as pain and trauma associated with daily, high level training and competition.

With a variety of different information types that can serve for inputs into data models, data mining and analysis will be able to identify those with the largest effects on performance, adaptation, fatigue, and injury risks, allowing for a more targeted approach to be taken to data collection if required. Furthermore, the integration of current technologies, such as urinary and salivary biomarkers (Lindsay & Costello, 2017), along with more standard physiological assessments, will likely enhance the predictive accuracy of these models.

The effectiveness of, and compliance to, a personalised training programme is currently unknown. In a pilot programme of 14 athletes, genetic information integrated into personalised injury prevention advice and techniques was found to reduce 12-month injury incidence, with more than half of the group finding the advice useful and implementing the recommendations (Goodlin et al., 2015). The use of optimisation software to determine pre-season training loads has proved successful in Australian Rules Football (Carey et al., 2018), whilst machine learning tools have been shown to outperform traditional methodologies in the prediction of response to training loads (Bartlett et al., 2017). Furthermore, a personalised approach to training has demonstrated effectiveness, with individualised training based on force-velocity profiling (Jimenez-Reyes et al., 2016) and HRV (Vesterinen et al., 2016) shown to enhance training adaptations. A major challenge will be to get athletes to accurately and consistently collect data—such as sleep metrics and HRV—away from the training field, with ease of use and lack of perceived invasiveness important factors for technology developers to consider in this regard. Nevertheless, despite these challenges, the development of a personalised training process appears to hold promise in the optimisation of athlete performance.

10. Summary

This chapter has explored the use of other novel technologies that, along with genetic information—the subject of this thesis—may combine in the development of the personalised training process. Whilst highly speculative and poorly researched, there is clearly scope for, and the acceptance of (Goodlin et al., 2015; Varley et al., 2018a), an increasingly personalised training process as a method to enhance athlete performance. An important aspect of such a model is that athlete adaptation and performance is highly complex, with a number of biological systems interacting to create the outcome. The purpose of this chapter is to demonstrate that, whilst genetic information likely does hold utility within elite athlete preparation, and likely does enhance the training process, it is crucial to keep in mind that genetic information represents only part of the picture. As research in this area grows, it should be

possible to achieve a better understanding of how genetics, and genetic variation, influence elite athlete performance, as well as how to best integrate the information from genetic testing within a holistic athlete preparation model, which in turn will enable a more personalised approach to athlete preparation. This could become highly complex, with the collection of large swathes of data to act as an input for complex predictive models, but there is a simple message contained within this; that each athlete has their own unique biology, and every day presents in a slightly different state—adaptive or maladaptive—that requires the coach to make changes on the fly. The better informed the coach is, the better the decisions he or she can make, but, more importantly, it is clear that all athletes should not be treated the same way, with one size fits all training programmes planned months in advance. Despite the potential complexity of a personalised training process, perhaps the simplest message—that all athletes need to be treated differently—is the most important.

CHAPTER 15: DISCUSSION – IS THERE UTILITY TO GENETIC INFORMATION IN ELITE SPORT?

1. Discussion

As stated in the introduction, the specific aim of this thesis was to explore the question "Is there utility to genetic information in elite sport?" Throughout this thesis, I believe that I have built the case that there is potentially significant utility for genetic information within elite sport, but, at present, more research is required to provide evidence-based guidelines to practitioners hoping to use genetic information to improve athlete performance—with some areas of this future research identified within the thesis.

In Section 2, which acted as my literature review, I explored the research around inter-individual variation in response to a training programme. Here, I suggested that three broad factors combine to determine the magnitude and type of exercise adaptations; genetic factors, environmental factors, and epigenetic factors (which can essentially be thought of as the effect of environmental influences on genetic expression). The interactions between these three aspects are highly complex and tangled; whilst, for example, consumption of vitamin D may modify the response to strength training (Chiang et al., 2017), variation in a number of genes can affect both baseline vitamin D levels, and the response to a vitamin D supplement (Didriksen et al., 2013); as such, many environmental factors are partially affected by genotype. Epigenetic modifications are also partially under the control of genetic factors; for example, variation in *MTHFR* may modify methylation efficiency (Nojima et al., 2018), which can affect post-exercise training adaptations (Terruzzi et al., 2011). Accordingly, it is clear that genetic variation is a consistent and fundamental modifier of variation in response to a stimulus—including exercise training—indicating that there the potential that understanding of an individual's genotype may assist in developing training programmes they are best able to adapt to.

Of course, the quality of scientific evidence within a field is only as good as the quality of the underlying studies, and in Chapters 3 and 4 I cast a critical eye over some of the outstanding issues within the exercise genomics literature. First, I discussed just how applicable and appropriate the term "non-responder", which is increasingly becoming part of the modern exercise scientist and coach's vocabulary, is. The main conclusion from that section was that exercise non-response is most likely a misnomer; by increasing the number of variables measured, as well as increasing training intensity, duration, and frequency, exercise non-response can be abated. Accordingly, it is my belief that there are no global non-responders to exercise, which is clearly good news from a public health perspective. Secondly, I asked whether, in many of the exercise genomics studies, we are truly finding what we think we're finding; i.e., are the results signal, or noise? There are many examples of genetic variants that appear to have one effect, but potentially have another; for example, the CC genotype of *COL5A1* has been associated with a reduction in exercise-associated muscle cramps (EAMC) (O'Connell et al., 2013). However, the same study also reported that CC genotypes were slower over a half-marathon run; as such, it's not clear whether *COL5A1* is directly protective against EAMC, or, given that neuromuscular fatigue is associated

with increased EAMC (Bergeron, 2008), the slower athletes (i.e. the CC genotypes) were protected against cramp by virtue of less fatigue. Aspects of my literature review were turned into three papers, published in *Sports Medicine* (Pickering & Kiely, 2018b), *BMJ Open Sports & Exercise Medicine* (Pickering & Kiely, 2017b), and *The Open Sports Science Journal* (Pickering & Kiely, 2017a).

In Chapter 4, I explored the history of exercise genetics research from twin studies, to candidate gene analysis, to genome-wide association studies. I wrote that, whilst current research is able to determine the impact of a specific genetic variant on sporting related traits, such as elite athlete status, training adaptations, and injury risk, at present there was a lack of research exploring how best to utilise this information. I proposed that a promising method to increase the utility of genetic information in the real-world would be the formation of total genotype scores (TGS), where a number of genetic variants are combined into a single output or score. I took this approach in the experimental section of the thesis (Chapters 10-12), where I utilised TGSs comprised of 5, 7, 48 and 64 genetic variants respectively; the latter two representing the most comprehensive TGS within the exercise genetics literature to date.

In Section 3 (Chapters 5-8), I attempted to "join the dots", providing an overview of how it might be possible to use genetic information to inform ergogenic aid use (Chapter 5), hamstring injury risk and prevention (Chapter 7), and talent identification (Chapter 8). These sections were subsequently published as papers in journals such as *Sports Medicine* (Pickering & Kiely, 2018a), *Medical Hypotheses* (Pickering & Kiely, 2018c), *Genes* (Pickering et al., 2019b), and *Sports Medicine Open* (Pickering & Kiely, 2017c). In Chapter 6, I also explored the wider implications of *ACTN3*—perhaps the gene most well-researched in terms of elite athlete status—to determine how it might affect training adaptations, post-exercise recovery speed, and injury risk. This was published in *Frontiers in Physiology* (Pickering & Kiely, 2017d), representing the first review article to explore this topic; more recent reviews have developed this topic further (Del Coso et al., 2018b; Houweling et al., 2018; Pickering & Kiely, 2018d). These studies have added to the present body of knowledge by demonstrating how genetic information might be effectively utilised within sport, pulling together previous research into a coherent analysis, and allowing practitioners to better understand the relative impact of genetic variation on a number of important aspects.

Section 4 (Chapters 9-12) then provided the experimental aspect of the thesis. Chapter 9 was a questionnaire which aimed to better understand the prevalence of, and attitudes towards, genetic testing within sport. Two hundred and fifty-six participants responded to the survey, consisting of 110 current or former athletes, of which 51% had competed internationally, and 133 members of support staff, of which 54% stated they most frequently worked with international athletes. Whilst the overall prevalence of genetic testing within sport at present is low (~10%), most participants stated that they believed genetics had a sizeable (>25%) effect on an athlete's chance of being an elite athlete, the adaptive response to training, and nutritional requirements. One of the main barriers to the use of genetic testing within sport is a perceived lack of evidence (cited by 39% of support staff), demonstrating the importance of an increased amount of research studies exploring the use of genetic information in sport, including both intervention and randomised controlled trials. This chapter adds to the current body of knowledge by quantifying the prevalence of genetic information within sport, building upon an earlier initial study by

Varley and colleagues (2018a) by increasing the sample size, and widening the number of sports explored. Furthermore, this study represents the first to explore the perceived barriers to the use of genetic information at this level.

In Chapter 10, I utilised a five SNP TGS in attempting to identify those athletes who would be expected to see larger improvements in aerobic fitness following an eight-week training block, and those expected to see smaller improvements. Using the results from the five SNP test, the players were stratified into three groups; “low”, “medium”, and “high”. There were no differences in Yo-Yo test performance between the groups at baseline; however, following an eight-week small-sided-games training programme, those in the “high” group had demonstrated significantly greater improvements in Yo-Yo performance than those in the “low” group. This suggests that those in the “low” group may be better suited to a different training intervention, although further work is required to determined what that intervention might be. This chapter was subsequently published in *PLoS One* (Pickering et al., 2018). This study is the first to use a commercially available panel of genes to predict the magnitude of response following an aerobic training intervention in sportspeople. Previous work (Timmons et al., 2010) has demonstrated that such an approach may be efficacious, but this had not been tested in a sporting population. Furthermore, the use of a readily available panel of genes is potentially advantageous as it represents a tool available to practitioners; as discussed in Chapter 4, one of the main issues in utilising genetic information in sport is a lack of validated multi-SNP panels. This study goes some way to addressing this information deficit.

In Chapter 11, I used the results of a seven SNP TGS to determine whether players were expected to exhibit a faster or slower time-course of recovery following a repeated sprints session. The players in the faster recovery genotype score group demonstrated smaller reductions in countermovement jump height immediately post-training, and at 24- and 48-hours post training. The effect sizes for these time points ranged from 0.5 (medium) to 1.0 (large), suggesting that the predictive ability of this algorithm is potentially useful, although the real-world utility was somewhat unclear. Similar to Chapter 10, this study is the first to utilise a commercially available panel of genes as a method to identify differences in recovery speed between individuals. Whilst previous studies (Del Coso et al., 2017a; 2018a) have utilised polygenic models to determine the magnitude of post-exercise muscle damage, the time-course of recovery was not determined in these studies. As a result, the study detailed in Chapter 11 represents the first study, to my knowledge, to use a polygenic score to explore the time course of post-exercise recovery, which could have large implications for practice in elite sport.

Finally, in Chapter 12, I explored whether a TGS comprised of 48 (power) or 64 (endurance) SNPs could discriminate between five elite athletes and 503 Caucasian non-athletic controls. Whilst the elite power athletes in the cohort—including an Olympic Champion—scored higher than the elite endurance athletes on the power score, they were outscored by 68 non-athletic controls. Conversely, the elite speed athletes outscored the elite endurance athletes in the endurance TGS. These results suggest that, even when a very large number of genetic variants are combined into a TGS, the information gained is insufficient to discriminate elite athletes from non-athletic controls, demonstrating that genetic testing cannot be used as a talent ID tool at this point in time. This paper is currently submitted to a journal, and

is undergoing peer-review. This study utilised polygenic scores comprised of the largest number of genetic variants that I am aware of within a sporting context. Accordingly, it represents the most comprehensive TGS studied from the perspective of talent identification, and as such represents a significant contribution to the knowledge base in this area.

2. Strengths & limitations

As with many studies within the exercise genetics field, the main limitation pertaining to the experimental aspect of my thesis is a lack of participant numbers. For example, in the aerobic training study (Chapter 10) I recruited 42 participants, and in the recovery study (Chapter 11) just 18. Low participant numbers are a commonly cited criticism within exercise genomics research (Bouchard, 2015). However, it is also worth noting that one of the aims of this thesis was to translate existing research—often utilising single genetic variants to explain observed effects—into useable information for those involved in elite level sport. As such, these elite sports coaches and support staff will often be working with lower numbers of athletes; a first-team football squad could conceivably be comprised of just 18 members, whilst the first and reserve team pools could number 42. As such, the participant numbers utilised here, whilst low, represent the real-world application of research to practice, something that is often lacking (Buchheit, 2017), and also mirror the samples sizes utilised within other exercise genetics studies (Del Coso et al., 2017a; Erskine et al., 2013; Santiago et al., 2010). Furthermore, large participant numbers are often required in exercise genomics research as the effect size of any given genetic variant is often low; increasing the participant numbers enhances the statistical power of the study. However, within this thesis I have combined the number of genetic variants utilised (using 5 for Chapter 10 and 7 for Chapter 11), which increases the effect size expected from a given result, and, again, provides increased real-world utility.

Conversely, in Chapter 12, I recruited five elite athletes, and utilised the data of 503 non-athletic controls. This represents a large sample size—in part required due to the low number of elite athletes—which is a particular strength of that Chapter. Furthermore, the recruitment of five elite athletes is an additional strength, particularly when the examination of highly elite athletes (my cohort included an Olympic Champion) is uncommon within the literature. Additionally, the questionnaire study detailed in Chapter 9 recruited a large number of elite athletes, with more than 50% of the sample having competed internationally, along with coaches and support staff working at a similar level. Being able to determine the attitudes of these individuals represents a further strength of the present thesis, although the vast majority (~80%) of participants recruited were males.

3. Implications for future research

This thesis has, hopefully, demonstrated how genetic variation exists as a fundamental and consistent modifier of many aspects affecting elite performance, from the attainment of elite athlete status, to training adaptations, injury reduction, and ergogenic aid use. In the theoretical aspects of this

thesis (Chapters 5 to 8), I have formalised various hypotheses around how genetic information may impact aspects related to sports performance and training. An obvious next step is to test these hypotheses experimentally, allowing us to better understand:

- Can the early results demonstrating a modifying effect of *CYP1A2* on caffeine ergogenicity be replicated? Do CC genotypes truly find moderate doses of caffeine ergolytic, as per the work of Guest and colleagues (2018), or do they merely require manipulation of dose and timing (Pickering, 2018)? Are other SNPs, such as those found in *ADORA2A*, able to modify the ergogenic effects of caffeine? How do these SNPs modify performance-related aspects such as pre-competition anxiety post-competition sleep?
- Can we discover a greater number of SNPs associated with the development of elite athlete status? How do genetic variants affecting wider aspects, including psychological and skill acquisition processes, affect the attainment of elite athlete status? Will it be possible to develop Total Genotype Scores (TGS) that are able to discriminate between elite athletes and non-athletes, given that current TGS do not (Chapter 12)?
- Do the genetic variants identified in Chapter 7, such as *ACTN3*, modify the strength adaptations seen following a period of eccentric hamstring loading? Do genetic variants associated with muscle structure and fascicle length, such as *TTN*, modify changes in these aspects following eccentric hamstring training? Is it possible to use this information to modify training programmes, with particular reference to managing the muscle damage and inflammatory response to damaging eccentric contractions? Are we able to use genetic information to "predict" injury susceptibility, and therefore reduce that risk prior to injury occurring?
- Can we use genetic information to modify training programme design, and drive increased adaptations? Whilst evidence suggests that genetic variants *explain* the inter-individual variation present in response to a training programme, far less research explores *using* this information to make changes to future training programmes.

Chapters 9-12 of this thesis explore the use of genetic information in sport experimentally, again raising additional questions for exploration:

- In Chapter 9, it was determined that one of the main perceived barriers to the use of genetic information within sport is that of a lack of evidence base as to both its utility, and its practical application. As such, a key aim for researchers moving forward should be to better understand whether and how genetic information might be used within sporting teams. Furthermore, many of the ethical considerations identified within that chapter require rectification before genetic testing can be widely adopted; researchers, bioethicists, and practitioners in the field need to work together to explore best practice guidelines aimed at protecting the athlete from potential harm and exploitation.
- Chapter 10 details the association of genetic information with the magnitude of post-exercise adaptations in terms of aerobic fitness. Can these results be replicated? Does the addition of other genetic variants tentatively associated with improvements in aerobic fitness, such as those identified by Williams and colleagues (2017), further strengthen the predictive ability of the current panel? Can those predicted to exhibit the smallest improvements be given alternative training methods in order to better enhance their fitness and/or performance?

- Chapter 11 demonstrates the potential utility of a seven SNP panel in predicting the time course of post-exercise recovery. As per the above point, can these results be replicated? Does the addition of further genetic variants to this panel improve its predictive ability? How can this information be used in the real-world?
- Finally, Chapter 12 explores the use of genetic information in talent identification, finding it to be ineffective. Can this ineffectiveness be resolved by increasing the number of genetic variants tested for? Does a weighted as opposed to unweighted TGS perform better in this regard? Can an increased number of genetic variants improve the predictive power of genetic information, such that it could be used in practice? Should genetic information—even if effective—ever be used as a talent identification tool?

Clearly, the work of this thesis has presented some additional questions, which is to be expected given the evolving nature of the subject matter. The resolution of some of these outstanding questions should go some way to enhancing the understanding of whether, and how, genetic information can best be utilised within sport, and provide evidence-based guidelines pertaining to its use. Given that a common criticism of genetic testing, particularly within sporting domains, is its lack of evidence base (Webborn et al., 2015), and that this is also a commonly cited reason for its lack of use in elite sport, such outcomes potentially represent a priority for researchers in this area.

4. Potential wider applications

Whilst those involved in sport are most often interested in increasing the performance of already high-level athletes to that required for elite performance, exercise also has a crucial role to play in the maintenance of optimal health (Pareja-Galeano et al., 2015), including disease prevention and management (Latino-Martel et al., 2016), as well as the maintenance of function—both physical and cognitive—with aging (Kuh et al., 2014). As a result, it is important to consider how breakthroughs at the level of elite sport might filter down to impact practice in this area. In Chapter 13, I explored this in extended detail across three parts.

Part One was an exploration of how *ACTN3*, the most well-researched gene in regard to elite athletic performance (Ma et al., 2013), might modify the healthy aging process. This section was turned into a paper, which was published in *Frontiers in Genetics* (Pickering & Kiely, 2018d), and was later built upon by Houweling and colleagues (2018). In summary, I concluded that *ACTN3* appeared to play a robust role in the maintenance of muscle mass and function with aging, and was also implicated in the maintenance of bone mineral density, although this was potentially due to the maintenance of physical function seen in R allele carriers. I also speculated that knowledge of *ACTN3* genotype had the potential, in future, to inform risk-management and risk-reduction strategies for attenuating sarcopenia and osteoporosis in elderly populations, as well as in the development of optimal training programmes to minimise this risk, either alone or in combination with other genetic variants, as per the work of Jones and colleagues (2016).

Part Two of Chapter 13 introduced the term "Exercise Response Efficiency", which relates to the ability of an individual to respond to a given exercise stimulus. Despite the fact that exercise has such broad and wide-ranging disease protective effects (Piepoli 2005; Fiuza-Luces et al., 2013; Pareja-Galeano et al., 2015; Sanchis-Gomar et al., 2015), many individuals do not meet the minimum guidelines, with some undertaking no exercise whatsoever (Ladabaum et al., 2014). The concept of exercise response efficiency refers to the matching of individuals to the type of exercise they are most likely to demonstrate the largest improvements from, with the suggestion being that this type of exercise will drive the greatest reductions in disease risk, along with increasing motivation through early positive changes, and hence have a large influence on public health. This part of the chapter is published as a paper in *Lifestyle Genomics* (Pickering & Kiely, 2019b). In Part Three, I explored whether genetic information may positively influence dietary management, which suggests that genetic information may enhance dietary adherence.

5. Implementation of genetic information into the athletic preparation process

Finally, in Chapter 14, I attempted to place genetic information into the context of the complete athlete preparation process, particularly in reference to a variety of emerging technologies. Whilst, as demonstrated in Chapters 2-12 of this thesis, there is utility to genetic information in elite sport, with genetics appearing to be a consistent and fundamental modifier of the training response, genetic information itself is static; a genetic test performed on the embryo of an athlete would return the same results as one carried out one the last scrap of biological material on that athlete's body long since they became deceased. As such, it's important to explore the wider use of genetic information in sport alongside other more plastic metrics, such as wellness markers or blood data. By combining all these pieces of information into a single model, we better understand the value of genetic information in the real world—that it represents a small, but potentially important, piece of information that can enhance athletic preparation. This chapter has been published as a paper in the *Journal of Functional Morphology and Exercise Kinesiology* (Pickering & Kiely, 2019a).

6. Real-World Impact

As identified in the introduction, the majority of research in the field of exercise genomics tends to focus on explaining what has previously happened, as opposed to attempting to use this information to better enhance the outcomes for athletes. One of my aims in undertaking this professional doctorate was to increase the depth of scientific research exploring the use of genetic information in this way. As a result, 13 publications have resulted directly from this thesis (detailed in table 11). A further paper is currently submitted to a journal and undergoing peer-review. As a result, I believe I have been successful in increasing the base of knowledge in this field.

Chapter	Paper	Comments
Chapter 2 - Inter-subject variation in exercise adaptation: Contributing factors & the potential utility of genetic testing	Pickering C, Kiely J. Understanding Personalized Training Responses: Can Genetic Assessment Help? Open Sports Sci J. 2017;10(1).	
Chapter 3 - Contemporary issues regarding exercise non-response and exercise genomics	Pickering C, Kiely J. Do non-responders to exercise exist—and if so, what should we do about them? Sports Med. 2018; https://doi.org/10.1007/s40279-018-01041-1	
Chapter 3 - Contemporary issues regarding exercise non-response and exercise genomics	Pickering C, Kiely J. Exercise genetics: seeking clarity from noise. BMJ Open Sport Exerc Med. 2017;3(1).	
Chapter 5 - Are the current guidelines on caffeine use in sport optimal for everyone? Inter-individual variation in caffeine ergogenicity, and a move towards personalised sports nutrition	Pickering C, Kiely J. Are the current guidelines on caffeine use in sport optimal for everyone? Inter-individual variation in caffeine ergogenicity, and a move towards personalised sports nutrition. Sports Med. 2018;48(1):7-16.	Twenty-nine citations, including an additional review on the effects of genetics on the individual response to caffeine (Southward et al., 2018), and one on the use of personalised nutrition from the BMJ (Ordovas et al., 2018).
Chapter 5 - Are the current guidelines on caffeine use in sport optimal for everyone? Inter-individual variation in caffeine ergogenicity, and a move towards personalised sports nutrition	Pickering C. Caffeine, CYP1A2 genotype, and sports performance: is timing important? Ir J Med Sci. 2018; doi: 10.1007/s11845-018-1811-4.	
Chapter 6 – *ACTN3*: More than just a gene for speed	Pickering C, Kiely J. ACTN3: More than just a gene for speed. Front Physiol. 2017;8:1080.	Six citations, including a more recent review (Del Coso et al., 201b8).

Chapter 7 – Genes, hamstring injury, and the response to eccentric training	Pickering C, Kiely J. Hamstring injury prevention: A role for genetic information? Med Hypotheses. 2018;119:58-62.	
Chapter 8 – Can genetic testing identify "talent" (whatever that might be)?	Pickering C, Kiely J. Can the ability to adapt to exercise be considered a talent—and if so, can we test for it? Sports Med Open. 2017;3(1):43.	
Chapter 8 – Can genetic testing identify "talent" (whatever that might be)?	Pickering C, Kiely J, Grgic J, Lucia A, Del Coso J. Can genetic testing identify talent for sport? Genes. 2019b;10(12):972.	
Chapter 10 – The magnitude of Yo-Yo test improvements following an aerobic training intervention are associated with total genotype score	Pickering C, Kiely J, Suraci B, Collins D. The magnitude of Yo-Yo test improvements following an aerobic training intervention are associated with total genotype score. PloS One. 2018;13(11):e0207597.	
Chapter 13 – Wider implications: Genetic information from a public health perspective	Pickering C, Kiely J. ACTN3, Morbidity, and Healthy Aging. Front Genet. 2018;9:15.	Cited by an additional review exploring the impact of *ACTN3* on human health and ageing (Houweling et al., 2018).
Chapter 13 – Wider implications: Genetic information from a public health perspective	Pickering C, Kiely J. Exercise Response Efficiency – A novel way to enhance population health? Lifestyle Genom. 2019.	
Chapter 14 – The implementation of genetic information within a personalised training framework	Pickering C, Kiely J. The Development of a personalised training framework: Implementation of emerging technologies for performance. J Functional Morphol Kinesiol. 2019;4(2):25.	

Table 11 – Publications arising directly from this thesis

Furthermore, my work has achieved attention in the lay press. During the course of the writing of this thesis, I have appeared on two TV programmes to discuss the potential utility of genetic testing for general health; BBC's Trust Me I'm a Doctor (December 2017), and ITV Tonight (January 2018). Additionally, I was requested to write articles summarising my research for the BMC blog network "On Medicine" (https://blogs.biomedcentral.com/on-medicine/2017/11/30/a-better-approach-to-talent-identification/), and the website "Science Trends" (https://sciencetrends.com/can-genetic-information-help-prevent-hamstring-injury/). Finally, as a direct result of some of the work contained within this thesis, I have been invited to meet with, and in some cases, directly support, a number of elite sporting teams (https://www.independent.co.uk/sport/football/international/mohamed-salah-liverpool-goals-olympics-craig-pickering-dnafit-gene-mapping-a8266576.html). As such, the work contained within this thesis has been impactful, both from an academic and real-world perspective, and my hope is to build on this in the coming years.

7. Conclusion – Is there utility to genetic information in elite sport?

In pulling the various strands of this thesis together, the main findings are that:

1. Genetic variation is a fundamental and consistent modifier of the response to a given stimulus. As such, genetic variation helps to explain the demonstrated differences in terms of training response, ergogenic aid effectiveness, injury risk, and the chances of becoming an elite athlete.
2. At present, many high-level athletes and support staff understand this genetic influence on a variety of sporting outcomes. However, approximately only 10% of athletes have undertaken a genetic test, with one of the main reasons cited for not utilising such tests in sport being a lack of evidence supporting their use.
3. Accordingly, research within the exercise genetics sphere needs to focus not just on explaining the observed variation in response to a stimulus, but on how to use this information to modify training- and lifestyle-based parameters in order to enhance athlete performance.
4. The grouping together of genes associated with a specific trait appears to improve the utility of a genetic test. In this thesis, Total Genotype Scores were able to determine participants likely to exhibit greater improvements in aerobic fitness following a training programme, as well as those expected to have increased recovery times following an exercise bout.
5. However, the creation of a Total Genotype Score comprised of a large number of genetic variants was not able to successfully discriminate a cohort of elite power and endurance athletes from non-athletic controls. As a result, there is no evidence, at present, that genetic information can be used to identify future talented performers.

At the start of this thesis, I asked whether there was any utility to genetic information within elite sport. Based on the findings reported throughout, and detailed above, I believe that it is clear that there is a strong potential utility of genetic information within elite sport, and, as such, genetic profiling has the

potential to improve sporting performance. In 2014, Williams and colleagues asked if we were at the starting line regarding the use of genetic information within sport. I believe that the gun has now fired, and we are making our first tentative steps towards the finish line. The outcomes of Sections Two and Three of the present thesis suggest there is a clear theoretical basis for the use of genetic information in sport. The results of Section Four, the practical part of this thesis, provide some evidence for how this would work in practice. Finally, Chapter 14 explores how genetic information may integrate along with other technologies, both emerging and current, to enhance athletic preparation. As future research develops and expands upon the findings of this thesis, evidence-based guidelines as to the use of genetic information within sport should evolve, further driving the field forwards, and assisting athletes and their support staff towards their common goal of enhancing performance.

ETHICS

The experimental studies carried out for this thesis, and detailed in Chapters 9-12, were carried out following ethics board approval, in line with the Declaration of Helsinki. The ethics board numbers were BAHSS 575, BAHSS 230, and SFEC 2016-020.

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APPENDIX 1 – QUESTIONS GIVEN TO ATHLETES AS PART OF THE STUDY DETAILED IN CHAPTER 9

1. I am an:

a. Athlete (continue to Q2)

b. Member of the support staff (coach, sports science, medicine) (go to support staff questionnaire)

2. What is your sex?

a. Male

b. Female

3. . Which sport are you primarily involved in?
    a. Football / Soccer
    b. Rugby (League / Union)
    c. Australian Rules Football
    d. American Football
    e. Basketball
    f. Ice Hockey
    g. Athletics (Track & Field, Road Running, Cross Country)
    h. Swimming
    i. Golf
    j. Racquet Sports (Tennis, Badminton)
    k. Baseball
    l. Winter Olympic Sports
    m. Other (please state)

4. What is your highest level of competition?
    a. Olympic Games or World Championships (including World Cup)
    b. International (you have represented your country)
    c. National (you have competed at the highest level within your country; i.e. national championships or top league).
    d. Regional (you have competed at county level, or in a league outside of the top league)
    e. Below-Regional / Recreational

5. What is your age range?
    a. <25 years old
    b. 26-35 years old
    c. 36-45 years old
    d. 46-55 years old
    e. 56-65 years old
    f. >66 years of age

6. Which region are you based in?
    a. UK / Ireland
    b. Russia
    c. Europe (not including the UK, Ireland, or Russia)
    d. USA
    e. North America (not including USA)
    f. South America
    g. Africa
    h. Middle East
    i. Australia and New Zealand
    j. Asia
    k. Other (please specify)

7. What is your highest level of completed education?
    a. High School (GSCE/A-level equivalent)
    b. University Undergraduate (e.g. BSc)
    c. University Post-Graduate (e.g. MSc)
    d. Doctorate (e.g. PhD)
    e. Other (please specify)

8. What impact do you think an individual's genetic make-up has on their chances of being an elite athlete?

a. None
b. Minimal (<25%)
c. Somewhat (25-75%)
d. Almost entirely (75%+)

9. What impact do you think an individual's genetic make-up has on their sporting/fitness improvements following exercise?

a. None
b. Minimal (<25%)
c. Somewhat (25-75%)
d. Almost entirely (75%+)

10. What impact do you think an individual's genetic make-up has on their nutrition requirements?
    a. None
    b. Minimal (<25%)
    c. Somewhat (25-75%)
    d. Almost entirely (75%+)

11. Have you ever used a genetic test targeted at sports performance?
        a. Yes (go to Q15)

b. No (go to Q12).

12. Why haven't you used genetic testing (please select all that apply)
    a. Too expensive
    b. Didn't know it existed
    c. Insufficient evidence
    d. Concerns about data protection
    e. Concerns about negative press coverage
    f. Ethical issues
    g. Other (please specify)

13. Do you envision using genetic testing in the future?
    a. Within the next year?
    b. Within the next 5 years
    c. Within the next 10 years
    d. Never

14. What would cause you to consider genetic testing (please select all that apply).
    a. Publication of case-studies
    b. Increased number of scientific studies utilizing genetic testing
    c. More teams/athletes using it
    d. Increased advertising and greater awareness of product
    e. Lower price
    f. Other (please specify)

All answers; go to end of Questionnaire

15. If you have used genetic testing, what was the main purpose of this?
    a. To see what sport/event you should compete in
    b. To inform training programme design
    c. Injury prevention
    d. Nutrition
    e. Screening for disease risk
    f. Commercial agreement/sponsorship
    g. General interest
    h. Other (please specify)

16. Did you find the information you received from the genetic test useful?
    a. Yes
    b. No

17. Why didn't you find the information you received useful?
    a. Too generic

b. Not targeted at sports people
c. Incorrect
d. Results were hard to understand
e. Other (please specify)

18. Who carried out your genetic testing?
    a. University / Academic Institution
    b. Commercial Company
    c. Other (please specify)

19. Did you find the results of the genetic test easy to understand?
    a. Yes
    b. No

20. Did you receive additional support from the genetic testing provider to enable you to understand the report?

a. Yes, and it was helpful
b. Yes, but it wasn't helpful
c. No

21. Did you make any lifestyle, dietary or training-based changes based on the results of the genetic test?
    a. Yes
    b. No

22. What changes did you make?
    a. Lifestyle
    b. Diet
    c. Training
    d. Recovery
    e. Other
    f. Box for further details.

APPENDIX 2 – QUESTIONS GIVEN TO COACHES AND SUPPORT STAFF AS PART OF THE STUDY DETAILED IN CHAPTER 9

1. I am an:

a. Athlete (go to support athlete questionnaire)

b. Member of the support staff (coach, sports science, medicine) (Continue to Q2)

2. What is your sex?

a. Male

b. Female

3. Which sport are you primarily involved in?

a. Football / Soccer

b. Rugby (League / Union)

c. Australian Rules Football

d. American Football

e. Basketball

f. Ice Hockey

g. Athletics (Track & Field, Road Running, Cross Country)

h. Swimming

i. Golf

j. Racquet Sports (Tennis, Badminton)

k. Baseball

l. Winter Olympic Sports

m. Other (please state)

4. With which level of athlete do you most frequently work with?

a. Olympic Games or World Championships (including World Cup)

b. International (you have represented your country)

c. National (you have competed at the highest level within your country; i.e. national championships or top league).

d. Regional (you have competed at county level, or in a league outside of the top league)

e. Below-Regional / Recreational

5. What is your role within your sporting organisation?

a. Sports medicine

b. Physiotherapist

c. Sports coach

d. Strength & Conditioning coach

e. Sports Scientist

f. Nutritionist

g. Other (please specify)

6. What is your age range?

a. <25 years old
b. 26-35 years old
c. 36-45 years old
d. 46-55 years old
e. 56-65 years old
f. >66 years of age

7. Which region are you based in?

a. UK / Ireland
b. Russia
c. Europe (not including the UK, Ireland, or Russia)
d. USA
e. North America (not including USA)
f. South America
g. Africa
h. Middle East
i. Australia and New Zealand
j. Asia
k. Other (please specify)

8. What is your highest level of completed education?

a. High School (GSCE/A-level equivalent)
b. University Undergraduate (e.g. BSc)
c. University Post-Graduate (e.g. MSc)
d. Doctorate (e.g. PhD)
e. Other (please specify)

9. What impact do you think an individual's genetic make-up has on their chances of being an elite athlete?

a. None
b. Minimal (<25%)
c. Somewhat (25-75%)
d. Almost entirely (75%+)

10. What impact do you think an individual's genetic make-up has on their sporting/fitness improvements following exercise?

a. None
b. Minimal (<25%)
c. Somewhat (25-75%)
d. Almost entirely (75%+)

11. What impact do you think an individual's genetic make-up has on their nutrition requirements?

a. None
b. Minimal (<25%)
c. Somewhat (25-75%)
d. Almost entirely (75%+)

12. Have you ever used a genetic test within your sporting organisation?

e. Yes (go to Q16)
f. No (go to Q13).

13. Why haven't you used genetic testing within your organisation? (please select all that apply)

a. Too expensive
b. Didn't know it existed
c. Insufficient scientific evidence
d. Concerns about data protection
e. Concerns about negative press coverage
f. Concerns around whether it is ethical
g. Other (please specify)

14. Do you envision using genetic testing in the future?

a. Within the next year?
b. Within the next 5 years
c. Within the next 10 years
d. Never

15. What would cause you to consider genetic testing (please select all that apply).

a. Publication of case-studies
b. Increased number of scientific studies utilizing genetic testing
c. More teams/athletes using it
d. Increased advertising and greater awareness of product
e. Lower price
f. Other (please specify)

All answers; go to end of Questionnaire

16. If you have used genetic testing, what was the main purpose of this?

a. To see what sport/event you should compete in
b. To inform training programme design
c. Injury prevention
d. Nutrition
e. Screening for disease risk
f. Commercial agreement/sponsorship

g. Other (please specify)

17. Did you find the information you received from the genetic test useful?

a. Yes

b. No

18. Why didn't you find the information you received useful?

a. Too generic

b. Not targeted at sports people

c. Incorrect

d. Results were hard to understand

e. Other (please specify)

19. Who carried out your genetic testing?

a. University / Academic Institution

b. Commercial Company

c. Other (please specify)

20. Did you find the results of the genetic test easy to understand?

a. Yes

b. No

21. Did you receive additional support from the genetic testing provider to enable you to understand the report?

a. Yes, and it was helpful

b. Yes, but it wasn't helpful

c. No

22. Did you make any lifestyle, dietary or training-based changes based on the results of the genetic test?

a. Yes

b. No

23. What changes did you make?

a. Lifestyle

b. Diet

c. Training

d. Recovery

e. Other

f. Box for further details.